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(54) Title: METHOD AND APPARATUS FOR SELECTION AND EVALUATION OF SUBSTANCES IN TREATMENT OF BONE DISORDERS

(57) Abstract: A method of determining which substance to administer to a vertebrate, a method of screening test substances, a method of determining the effect of a substance and a data-base for holding such information. A method is disclosed for obtaining information relating to which parameter of e.g. an osteoporotic bone is to be affected by a substance. A data-base is generated wherein substances, having an effect on bone, are identified together with information on which bone parameters are affected by the substance. By coordinating information in the database, a substance or a combination of substances is provided for providing the desire effect on the bone. It has been found that especially cortical porosity is affected by Bisphosphonates, and especially cortical thickness is affected by HRT.

METHOD AND APPARATUS FOR SELECTION AND EVALUATION OF SUBSTANCES IN TREATMENT OF BONE DISORDERS

The present invention relates in general to different aspects of a more precise determination of which types of defects or conditions may take place or exist in bones and how to determine which substance or substances to administer in order to counteract such defects or conditions. A large group of such defects or conditions is seen as different types of osteoporosis.

Pharmacological agents used to manage osteoporosis can be divided according to their mechanism of action. Today two main groups exist, which are the antiresorptive agents and the bone formatting agents. Beside these two main groups there is a third group with agents having more heterogeneous effects such as the anabolic steroids, vitamin D and its analogues. Most of the agents that are commercial available today belongs to the antiresorptive group, which are believed to act by decreasing the rate of bone resorption and thereby slowing the rate of bone loss. Some of the agents have in large clinical studies shown to reduce fracture risk. The optimal timing and duration of treatment for most of the agents remain to be defined and will probably differ between them.

Below the different pharmacological agents, who may have a potential in relation to the management of osteoporosis, are grouped according to their mechanism of action. Only very few of the agents are approved for treatment of osteoporosis and most of them are still under development. Examples from each group are given together with the names of some of the producers.

Antiresorptive Agents

The antiresorptive agents are expected to act through a direct or indirect inhibition of the osteoclasts. The group may be divided into the following four classes:

Bisphosphonates

Etidronate (Proctor & Gamble, Gentili)

Clodronate (Astra, Boehringer Mannheim/Roche, Rhone-Poulenc Rorer)

Pamidronate (Novartis, Gador)

Alendronate (MSD, Gentili)

Risedronate (Proctor & Gamble, Takeda)

Ibandronate (Boehringer Mannheim/Roche)

Zoledronate (Novartis)

Tiludronate (Sanofi)

Incadronate (Yamanouchi)

Neridronate (Gentili)

Olpadronate (Gador)

EB-1053 (Leo)

YH 529 (Yamanouchi - Hoechst Marion Roussel)

Hormone Replacement Therapy

Estrogens (Novartis, Novo Nordisk, Organon and others)

Estrogens with progestogens (Novartis, Novo Nordisk, Organon and others)

Tibolone (Organon)

Trimegestone (Hoechst Marion Roussel)

Selective Estrogen Receptor Modulator (SERM)

Raloxifene (Eli Lilly)

Droloxifene (Pfizer)

Idoxifene (SmithKline Beecham)

Levormeloxifene (Novo Nordisk)

Tamoxifen (Zeneca, Rhone-Poulenc Rorer and others)

Calcitonin

Salmon calcitonin (Novartis, Rhone-Poulenc Rorer, Leo and others)

Human calcitonin (Suntory)

Calcitonin gene-related peptide (Warner-Lambert)

The agents that so far have been approved for treatment of osteoporosis are nearly all belonging to the group of antiresorptive agents.

Agents with Heterogeneous Effect

The agents in this group have so far been less well characterised with respect to their mechanism of action on the bone.

Stanozolol
Oxandrolone
Nandrolone
Dihydrotachysterol and other vitamin D derivatives
Alfacalcidiol
Calciferol
Calcitriol (Roche)
Cholecalciferol
ST-630 (Discovery Laboratories)
Calcium (Novartis and others)
Calcium with vitamin D (Nycomed and others)
Ipriflavone and other isoflavones
Strontium (Servier)
Osteoprotegerin (OPG) (Amgen)

The Bone Formatting Agents

The bone formatting agents are expected to act through a direct or indirect stimulation of the osteoblasts through a mitogenic mechanism. So far only the fluorides have extensively been used in the treatment of osteoporosis. Examples of bone formatting agents are given below:

Sodium fluoride (Mission Pharmacal)
Monofluorophosphate
Parathyroid hormone$_{1-84}$ (Eli Lilly)

Parathyroid hormone$_{1-34}$ (Allelix Biopharmaceuticals)
Growth hormone (Novo Nordisk, Eli Lilly, Pharmacia & Upjohn and others)
Growth hormone releasing compounds (Novo Nordisk and others)
IGF-1 (Pharmacia & Upjohn, Novartis)
IGF-BP3 (Celtrix Pharmaceuticals)
Osteogenic potein-1 (Creative BioMolecules)
Bone morphogenetic protein-2 (Genetics Institute, Yamanouchi)

Because osteoporosis is associated with low bone mass, traditional bone mineral density (BMD) measured by Dual-energy X-ray absorptiometry (DXA) are used to assess the efficacy of treatment. However, there is growing evidence that BMD is just one of many factors that contribute to the bone strength. Clinical studies have demonstrated that there is no simple relationship between changes in BMD induced by treatment and changes in fracture incidence. In studies with sodium fluoride, BMD at the lumbar spine increased by 35% but there was no decrease in fracture incidence. Bisphosphonates have been shown to increase bone mass by about 10 %, which was associated with a reduction in fracture incidence of 50%. However, on the basis of a simple mass-strength correlation an increase of 10% in BMD after bisphosphonate treatment should only result in an increase in strength of 15% and, presumably, a corresponding decrease in fracture incidence. Also in relation hormone replacement therapy (HRT) and calcitonin significant reductions in fracture incidence have been demonstrated despite relatively small changes in BMD. The results from several clinical studies have shown that fracture incidence can be reduced significantly with only a small increase in BMD, and that this discrepancy may be explained by an effect of treatment on the bone micro-architecture.

Bone micro-architecture can be evaluated though histomorphometry based on biopsies drilled out of the ilium bone. This method is very costly and time consuming and also inconvenient to the patients and therefore only applicable in small experimental studies. It has been suggested that quantitative computed topography (QCT) could be used to assess changes in the bone quality, but due to the high precision error with this technique it is presently not usable for follow-up measurements. Further the QCT devices are very expensive and also the higher x-ray dose limits the use for routine measurements. Besides, looking at the fracture incidence, the only non-invasive technique available today in relation to assessing the effect of an anti-osteoporotic treatment is by measuring BMD. A newly developed method termed Digital X-ray Radiogrammetry (DXR) provides more

relevant and detailed information about treatment effect than can be obtained with conventional DXA technology. Data from a recent pilot study seems to indicate that the qualitative bone parameters generated by DXR is more sensitive than DXA to changes in the bones following anti resorptive treatment. Further, the DXR technology is also expected to be able to distinguish between the individual anti resorptive agents with respect to their pharmacological effects on the bones.

Only a few investigations have been performed in order to determine the actual effect of such substances on specific parameters. Normally, these investigations entail firstly providing a certain condition in a large number of test animals and subsequently administering the substance. The actual determination of the effect on the bone is performed by sacrificing the animal, extracting the bone therefrom and cutting the bone in order to gain access to the interior structure thereof. This type of method may be sufficient in order to obtain a vague indication of the effect on the bone. However, it is not desired having to 1) perform the test on animals with artificially induced conditions, 2) to sacrifice the animal (and thereby not having the possibility of continuing the experiment on the same animal), 3) using a determination which requires removing the bone from the vertebrate.

According to the invention, a technique has been found that facilitates this more desired type of investigation. Also, it is now possible to both differentiate between different bone defects or conditions and to determine which substance or group of substances would act to counteract that defect of condition. Thus, a much more specific and precise method has been found - and a method which may be performed fully non-invasively and without destroying the bone and without harming the vertebrate. This also provides a better manner of investigating the effects of the substances in that inter-vertebrate differences are now less prominent in that measurements on the same vertebrate may be performed in the course of the investigation.

In a first aspect, the invention relates to a method of determining which substance, of a number of substances having an effect on bone, to administer to a vertebrate in need thereof, the method comprising:

- providing image data relating to one or more bones of the vertebrate,
- deriving, from the image data, one or more parameters relating to the bone,
- providing first information relating to the one or more parameters of the one or more bones of vertebrates classified as not suffering from bone diseases,

- comparing the first information and the derived one or more parameters and determining differences there between,
- providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby,
- determining which substance/substances to administer to the vertebrate on the basis of the result of the comparison and the second information.

An alternative to this first aspect is one relating to an apparatus for providing information relating to the effect of a substance on a vertebrate, the apparatus comprising:

- means for providing image data relating to one or more bones of the vertebrate,
- means for deriving, from the image data, one or more parameters relating to the bone,
- means for providing first information relating to the one or more parameters of the one or more bones of vertebrates classified as not suffering from bone diseases,
- means for comparing the first information and the derived one or more parameters and determining differences there between,
- means for providing second information relating to each of the number of substances and information relating to which of the one or more parameters of bones are affected thereby,
- means for determining which substance/substances to administer to the vertebrate on the basis of the result of the comparison and the second information.

Naturally, this apparatus may comprise means for performing any of the below mentioned steps and manners of processing the data and determining features therefrom.

In the present context, "in need thereof" would normally mean that the vertebrate has been diagnosed, by a physician or another expert, to not be in an optimal condition, such as if having a non-optimum bone mineral content, bone mineral density, fracture risk, bone mineral content, bone strength, bone quality or the like.

Thus, preferably, the method is performed on a vertebrate or vertebrates classified as suffering from Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes

(IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer or prostate cancer.

Conversely, bones "not suffering from bone diseases" would normally be bones, the condition (such as bone mineral density, fracture risk, bone mineral content, bone strength, bone quality or the like) of which is normal taking into account the weight, height, sex, ethnical background, age, menopausal status, life style and habits, family and/or medical history of the vertebrate.

Also, the first information would normally indicate normal or healthy values for the one or more parameters. Naturally, these values may vary between individuals and groups of individuals, such as dependent on weight, height, sex, ethnical background, age, menopausal status, life style and habits, family and/or medical history. It should be noted that this information might be derived from individuals, which are, in fact, not healthy, as long as they are not diagnosed as having bone diseases or conditions.

"not having received the substance" will normally mean that the substance has not been administered in amounts where the effect, which the vertebrate is not expected to show, is not pronounced - or preferably even detectable. Preferably, these vertebrates have not received the substance at all.

Normally the information relating to which of the one or more parameters are affected by which substances will at least comprise, for each parameter or each substance, the identity of which substance is affected or which parameter it affects, respectively. However, below is given an example of further information, which may be provided.

Also, the determination step may comprise deriving information on suitable doses or intervals of administration.

Preferably the deriving step comprises deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

The present physical distances in the bone may be any physical distance determinable from the image data. However, it should be noted that a distance determinable in the image data might not relate to an easily determinable distance in the bone in that the generation of the image data may alter distances in the bone. An example of this fact is the two-dimensional image data generated by a standard X-ray image of the bone. In this image, a distance may not correlate to the same distance in the bone in that the X-ray image is a projection of the three-dimensional bone structure onto two dimensions. In two-dimensional data, the physical distance would be a distance of e.g. a hard copy thereof or a metrical distance determinable between e.g. different parts of the image data.

A variation of a density of cortical and/or trabecular bone may be caused by a number of effects, such as the function of osteoclasts and osteoblasts in bone. These variations may be determinable from the image data in a number of manners. In the example where the image data are represented in grey values, these variations are determinable as grey value variations, such as areas having grey values different from surrounding areas. Again, such variations in the bone may not correspond directly to variations in the image data. However, a correlation will exist.

Bone diseases/effects/conditions may exist which affect a given parameter in one direction, and others may exist which affect the parameter in the opposite direction. These facts may point at different problems and illnesses and may be counteracted by different substances. Therefore, the second information may further comprise, for each substance and for at least one numerical parameter, information relating to whether the parameter is increased or decreased by the effect of the substance. In that situation, the given parameter(s) would normally comprise the at least one parameter and wherein the comparing means also comprise means for deriving information relating to whether a desired effect of the substance is to reduce or increase the parameter.

In this context, a numerical parameter will be a parameter, which is quantifiable with e.g. a number or digit. This will normally be the case with parameters derived by computers or other automatic measures.

The "desired effect" of the substance will be the effect desired in order to bring at least some of the one or more parameters to or toward the parameters of healthy vertebrates. In this manner, it will be determinable whether the at least one numerical parameter

should be decreased or increased in order to bring that or those parameter(s) to or toward those of healthy vertebrates.

Thus, the determining means may comprise means for determining a combination of substances providing the desired effect on the bone, where the determining means are adapted to take into account the information relating to the numerical parameter. Thus, when determining which substance or substances to administer, a single substance may not exist (or has not yet been identified) which counteracts the offset (difference from the parameter(s) of the first information) of the parameters sufficiently. Also, such a substance may exist but have the "side effect" that it also has an effect on a parameter, which is not to be affected in the bone. In those situations, a group of substances may be identified, the total effect of which is that desired and where e.g. "side effects" of one substance is counteracted by the effect of another.

The determining means may be adapted to provide, in relation to at least some of the one or more parameters, information relating to an uncertainty, such as a statistical uncertainty and/or an uncertainty deriving from the providing of the image data or the parameters, in the deriving of the parameter(s) and to, on the basis of that uncertainty, determine only parameter(s) significant in relation to their related uncertainties. Even though a parameter seems to vary quite significantly with a given effect, this may not be true taking the uncertainty into account. Thus, parameters, which are made less useful due to a large uncertainty, are then automatically not taken into account.

In a first situation, the step of providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby comprises, for a substance:

- providing first image data relating to at least part of a bone of a vertebrate at a first point in time,
- administering the substance to the vertebrate after the first point in time,
- providing, at a second point in time subsequent to the administering of the substance, second image data relating to the at least part of the bone,
- deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,

- comparing the one or more parameters relating to the first and second points in time, and
- providing, from the result of the comparison, information relating to which of the one or more parameters is affected by the substance.

Normally, the providing step will provide information relating to parameter(s) which, from the comparing step, is/are seen to vary with the administering of the substance. As is described above, this step may comprise evaluating a statistical uncertainty in order to provide information only on parameter(s) statistically significant.

Thus, image data are provided at different points in time in order for the determination to be able to build on measurements performed on the same vertebrate. Preferably, the image data relate to the same bone in the vertebrate. However, it is normally contemplated that at least a number of bone diseases or conditions act equally on all bones in a vertebrate, whereby different image data of different bones may, in fact, provide the information required.

Again, preferably, the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

The method of the first situation preferably further comprises administering the substance to the vertebrate a number of times between the first and second points in time and at predetermined doses and time intervals in order to ensure reproducible results.

Also, in order to be able to better follow the course of action in the bone, the method may further comprise providing third image data of the at least part of the bone and at least one third point in time between the first and second points in time, deriving, from the third image data, the one or more parameters, and comparing, in the comparing step, the one or more parameters relating to the first, second, and third points in time.

In order to increase the statistical certainty of the method, the image data may be provided of at least parts of bones of a number of vertebrates, where the substance is pro-

vided to the number of vertebrates, where the one or more parameters are derived from each of the vertebrates, and wherein the parameters of the vertebrates are compared in the comparing stepin order to provide information relating to an overall effect of the substance on bone statistical analysis of the parameters of the vertebrates.

For a number of known parameters, only image data relating to a part of the bone suffices in order to derive a value for each parameter.

A large number of bone disorders or conditions exist, and it is contemplated that some of these act differently on bones in the vertebrate and others more homogeneously. Normally, the vertebrate or vertebrates would be classified as suffering from bone disorders or conditions, such as type I and II osteoporosis, glucocorticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast and/or prostate cancer.

In a second situation, the step of providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby comprises, for a substance:

- providing a first group of vertebrates having received the substance,
- providing a second group of vertebrates not having received the substance to any significant degree,
- providing a first number of image data relating to at least part of the bone of each of the first group of vertebrates,
- providing a second number of image data relating to the at least part of the bone of each of the second group of vertebrates,
- deriving, from the first number of image data, one or more parameters relating to the bones of the first group of vertebrates,
- deriving, from the second image data, the one or more parameters relating to the bones of the second group of vertebrates,
- comparing the one or more parameters relating to the first and second groups of vertebrates and
- determining, from the result of the comparison, the effect of the substance on the bones of the vertebrates of the first group.

In the present context, "not having received the substance to any significant degree" will normally mean that the substance has not been administered in amounts where the effect, which the vertebrate is not expected to show, is not pronounced - or preferably even detectable. Preferably, these vertebrates have not received the substance at all.

Also in this situation is it preferred, that the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

Thus, instead of providing time-related information relating to e.g. a single vertebrate, information may be provided from a number of vertebrates where some have and some have not received the substance - but at the same point in time.

As described above, preferably the same bone is used in all vertebrates. However, this may not be required in all situations.

Different types of investigations of substances exist, in one of which the vertebrates of the first group of vertebrates would be classified to have or have been brought to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and wherein the vertebrates of the second group of vertebrates would be classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions.

The present classification will normally be performed by professionals, such as doctors or physicians. Also, professionals will typically be those actually bringing vertebrates to such states or conditions. It is a widely used method to bring a vertebrate to a condition being or closely resembling a given condition in order to test the effect of a given substance.

In fact, normally, the vertebrates, typically from animals, will not actually be brought to have the actual disease but will be brought to have what is meant to be a model thereof. Thus, instead of generating the actual disease, which may, in fact, be difficult, the vertebrate is brought to a state, which is believed to be a good approximation.

In another type of investigation, the vertebrates of the first and second groups of vertebrates would be classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions - and in that situation, the method may comprise:

- providing a third group of vertebrates, and wherein the vertebrates of the third group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions,
- providing a third number of image data relating to the bone of each of the third group of vertebrates,
- deriving the one or more parameters from the third image data, and
- comparing, in the comparing step, the one or more parameters of the first, second, and third groups of vertebrates.

Normally, the comparing step will comprise comparing the parameters for each vertebrate individually.

In any case, the disease or condition or group of diseases or conditions may be chosen from: Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast and/or prostate cancer.

Also, in order to be able to follow the development over time, the method may comprise providing the first, second and optionally third sets of image data at at least two different points in time between which the first group has received at least part of the substance received, and wherein the comparing step comprises comparing the image data obtained at the at least two points in time.

In a third situation, the step of providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby comprises, for a substance:

- providing first image data relating to at least part of a bone of a vertebrate at a first point in time,

- administering the substance to the vertebrate after the first point in time,
- providing, at a second point in time subsequent to the providing of the drug, second image data relating to the at least part of the bone,
- deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,
- comparing the one or more parameters relating to the first and second points in time,
- determining, from the result of the comparison, whether the substance has any effect on the bone and
- providing, if the substance has an effect on the bone, information that the substance has an effect on the bone and which of the one or more parameters is affected thereby.

Again, preferably the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

Preferably, the substance is administered to the vertebrate a number of times between the first and second points in time and at predetermined doses and time intervals. This is the most natural method of using the substance.

As is also described above, the method preferably further comprises providing third image data of the at least part of the bone and at at least one third point in time between the first and second points in time, deriving, from the third image data, the one or more parameters, and comparing, in the comparing step, the one or more parameters relating to the first, second, and third points in time.

Also, the image data are preferably provided of at least parts of bones of a number of vertebrates, where the substance is provided to the number of vertebrates, where the one or more parameters are derived from each of the vertebrates, and wherein the parameters

of the vertebrates are compared in the comparing step in order to provide information relating to an overall effect of the substance on bone.

In a fourth situation, the step of providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby comprises, for a substance:

- providing a first group of vertebrates having received the substance,
- providing a second group of vertebrates not having received the substance,
- providing a first number of image data relating to at least part of the bone of each vertebrate of the first group of vertebrates,
- providing a second number of image data relating to the at least part of the bone of each vertebrate of the second group of vertebrates,
- deriving, from the first number of image data, one or more parameters relating to the bones of the first group of vertebrates,
- deriving, from the second image data, the one or more parameters relating to the bones of the second group of vertebrates,
- comparing the one or more parameters relating to the first and second groups of vertebrates,
- determining, from the result of the comparison, whether the substance has any effect on the bones of the vertebrates of the first group and
- providing, if the substance has an effect on the bone, information that the substance has an effect on the bone and which of the one or more parameters is affected thereby.

Again, the vertebrates of the first group of vertebrates may have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and wherein the vertebrates of the second group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions.

Alternatively, the vertebrates of the first and second groups of vertebrates may have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and in that situation, the method preferably further comprises:

- providing a third group of vertebrates, and wherein the vertebrates of the third group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions,
- providing a third number of image data relating to the bone of each of the third group of vertebrates,
- deriving, from the third image data, the one or more parameters, and
- comparing, in the comparing step, the one or more parameters of the first, second, and third groups of vertebrates.

Also, in a second aspect, the invention relates to a method of differentiating between different types of bone lesions/defects/conditions, the method comprising:

a) providing image data on a bone in at least one vertebrate, which is suspected to suffer from a first bone disease or condition,

b) providing image data on a bone in at least one vertebrate, which is suspected to suffer from a second bone disease or condition

c) deriving one or more parameters relating to the bones based on said image data provided in step a) and b),

d) providing information relating to said one or more parameters of bones of vertebrates not suffering from the bone disease or condition,

e) comparing the one or more parameters obtained in step b) with the one or more parameters obtained in step c) to determine differences in said parameters, and

f) identifying, on the basis of the information provided in step d) and the result of the comparison of step e), one or more parameters relating to said bones of said vertebrates and being correlated to one or each of the first and second bone diseases or conditions.

That a vertebrate is "suspected" to suffer from a disease or condition will normally be derived by a professional, such as a doctor or a physician. The same will be the identification of vertebrates "not suffering" from the disease or condition.

"Being correlated to" will in this respect mean that a parameter "varies with the disease or condition": if the disease or condition is absent, the parameter is the same for that vertebrate and a healthy vertebrate - if not, it is different.

It is clear from the above that the present invention may not only provide useful information relating to how to counteract certain bone diseases, it also provides useful information relating to exactly how substances affect bones. It will be natural to compile such information in order to have a much more efficient use thereof.

In a third aspect, the invention relates to a method of determining the effect of a substance on a bone of a vertebrate, the method comprising:

- providing first image data relating to at least part of the bone at a first point in time,
- administering the substance to the vertebrate after the first point in time,
- providing, at a second point in time subsequent to the administering of the substance, second image data relating to the at least part of the bone,
- deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,
- comparing the one or more parameters relating to the first and second points in time, and
- determining, from the result of the comparison, the effect of the substance on the bone

wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

An alternative to this third aspect is one relating to an apparatus for determining the effect of a substance on a bone of a vertebrate, the apparatus comprising:

- means for providing first image data relating to at least part of the bone at a first point in time,
- means for providing, at a second point in time, second image data relating to the at least part of the bone,
- means for deriving, from the first image data, one or more parameters relating to the bone at the first point in time,

- means for deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,

the deriving means being adapted to derive, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone

- means for comparing the one or more parameters relating to the first and second points in time, and
- means for determining, from the result of the comparison, the effect of the substance on the bone.

Preferably, naturally, the first image data is provided at a point in time prior to that of the providing the second image data - and preferably, the bone has received the substance at at least one point in time between these two points in time.

This apparatus may comprise means for performing any of the below method steps related to the processing of the data.

Thus, image data are provided at different points in time in order for the determination to be able to build on measurements performed on the same vertebrate. Preferably, the image data relate to the same bone in the vertebrate. However, it is normally contemplated that at least a number of bone diseases or conditions act equally on all bones in a vertebrate, whereby different image data of different bones may, in fact, provide the information required.

The method preferably further comprises administering the substance to the vertebrate a number of times between the first and second points in time and at predetermined doses and time intervals in order to ensure reproducible results.

Also, in order to be able to better follow the course of action in the bone, the method may further comprise providing third image data of the at least part of the bone and at least one third point in time between the first and second points in time, deriving, from the third

image data, the one or more parameters, and comparing, in the comparing step, the one or more parameters relating to the first, second, and third points in time.

In order to increase the statistical certainty of the method, the image data may be provided of at least parts of bones of a number of vertebrates, where the substance is provided to the number of vertebrates, where the one or more parameters are derived from each of the vertebrates, and wherein the parameters of the vertebrates are compared in the comparing step.

For a number of known parameters, only image data relating to a part of the bone suffices in order to derive a value for each parameter.

A large number of bone disorders or conditions exist, and it is contemplated that some of these act differently on bones in the vertebrate and others more homogeneously. Normally, the vertebrate or vertebrates would be classified as suffering from bone disorders or conditions, such as type I and II osteoporosis, glucocorticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast and/or prostate cancer.

In yet another aspect, the invention relates to a method of determining the effect of a substance on a bone of a vertebrate, the method providing:

- providing a first group of vertebrates having received the substance,
- providing a second group of vertebrates not having received the substance to any significant degree,
- providing a first number of image data relating to at least part of the bone of each of the first group of vertebrates,
- providing a second number of image data relating to the at least part of the bone of each of the second group of vertebrates,
- deriving, from the first number of image data, one or more parameters relating to the bones of the first group of vertebrates,
- deriving, from the second image data, the one or more parameters relating to the bones of the second group of vertebrates,

- comparing the one or more parameters relating to the first and second groups of vertebrates and
- determining, from the result of the comparison, the effect of the substance on the bones of the vertebrates of the first group

wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

Thus, instead of providing time-related information relating to e.g. a single vertebrate, information may be provided from a number of vertebrates where some have and some have not received the substance - but at the same point in time.

In the present context, "not having received the substance" will mean that the substance has not been administered in amounts where the effect, which the vertebrate is not expected to show, is pronounced - or even detectable. Preferably, these vertebrates have not received the substance at all.

As described above, preferably the same bone is used in all vertebrates. However, this may not be required in all situations.

Different types of investigations of substances exist, in one of which the vertebrates of the first group of vertebrates would be classified to or have been brought to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and wherein the vertebrates of the second group of vertebrates would be classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions.

In fact, normally, the vertebrates, typically from animals, will not actually be brought to have the actual disease but will be brought to have what is meant to be a model thereof. Thus, instead of generating the actual disease, which may, in fact, be difficult, the vertebrate is brought to a state, which is believed to be a good approximation.

In another type of investigation, the vertebrates of the first and second groups of vertebrates would be classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions - and in that situation, the method may comprise:

- providing a third group of vertebrates, and wherein the vertebrates of the third group of vertebrates have been classified to not have the given disease or condition or a disease or condition within a given group of diseases or conditions,
- providing a third number of image data relating to the bone of each of the third group of vertebrates,
- deriving the one or more parameters from the third image data, and
- comparing, in the comparing step, the one or more parameters of the first, second, and third groups of vertebrates.

In any case, the disease or condition or group of diseases or conditions may be chosen from: Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast and/or prostate cancer.

Also, in order to be able to follow the development over time, the method may comprise providing the first, second and optionally third sets of image data at at least two different points in time between which the first group has received at least part of the substance received, and wherein the comparing step comprises comparing the image data obtained at the at least two points in time.

Also, in one aspect, the invention relates to a type of Drug Discovery, that is, a method of screening test substances to identify substances which have an effect on a bone of a vertebrate, the method comprising:

- providing first image data relating to at least part of the bone at a first point in time,
- administering the substance to the vertebrate after the first point in time,
- providing, at a second point in time subsequent to the providing of the drug, second image data relating to the at least part of the bone,

- deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,
- comparing the one or more parameters relating to the first and second points in time, and
- determining, from the result of the comparison, whether the substance has any effect on the bone

wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone

Preferably, the substance is administered to the vertebrate a number of times between the first and second points in time and at predetermined doses and time intervals. This is the most natural method of using the substance.

As is also described above, the method preferably further comprises providing third image data of the at least part of the bone and at at least one third point in time between the first and second points in time, deriving, from the third image data, the one or more parameters, and comparing, in the comparing step, the one or more parameters relating to the first, second, and third points in time.

Also, the image data are preferably provided of at least parts of bones of a number of vertebrates, where the substance is provided to the number of vertebrates, where the one or more parameters are derived from each of the vertebrates, and wherein the parameters of the vertebrates are compared in the comparing step.

In a further aspect, the invention relates to a method of screening test substances to identify substances which have an effect on a bone of a vertebrate, the method comprising:

- providing a first group of vertebrates having received the substance,
- providing a second group of vertebrates not having received the substance,

- providing a first number of image data relating to at least part of the bone of each vertebrate of the first group of vertebrates,
- providing a second number of image data relating to the at least part of the bone of each vertebrate of the second group of vertebrates,
- deriving, from the first number of image data, one or more parameters relating to the bones of the first group of vertebrates,
- deriving, from the second image data, the one or more parameters relating to the bones of the second group of vertebrates,
- comparing the one or more parameters relating to the first and second groups of vertebrates and
- determining, from the result of the comparison, whether the substance has any effect on the bones of the vertebrates of the first group

wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

Again, the vertebrates of the first group of vertebrates may have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and wherein the vertebrates of the second group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions.

Alternatively, the vertebrates of the first and second groups of vertebrates may have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and in that situation, the method preferably further comprises:

- providing a third group of vertebrates, and wherein the vertebrates of the third group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions,
- providing a third number of image data relating to the bone of each of the third group of vertebrates,
- deriving, from the third image data, the one or more parameters, and

- comparing, in the comparing step, the one or more parameters of the first, second, and third groups of vertebrates.

It may be desired that each vertebrate be a laboratory or test animal, and wherein the method comprises finally sacrificing the animal in order to e.g. be able to perform more advanced studies of the vertebrate subsequent to the other parts of the method.

Independently on which of the aspects of the invention is used, a number of methods are known for providing image data relating to bones. Each of these methods has advantages and disadvantages - such as the obtainable resolution, the minimum radiation dose required, etc.

It is presently preferred to acquire information relating to the internal structure of the bone - and in a manner where the bone is not destroyed, the image data are preferably obtained by a non-invasive method, such as by exposing the bone or bones to radiation, such as X-rays, performing a CT scanning, a NMR scanning or exposing the bone or bones to ultra sound.

In order to obtain reasonable calculation speeds, the image data are preferably two-dimensional image data relating to at least part of the bone or bones. Also, depending on the actual method, only a part of a single bone, such as ultra sound on the heel, is used, and in other methods, such as X-ray of a hand, several bones may be seen in the image data.

A vast number of parameters may be derived from the above methods. Parameters of these types may be seen in e.g. WO 96/07161, WO 97/42602, and WO 99/01835 - all of which are hereby incorporated by reference.

One situation is one where at least one of the one or more parameters relates to a physical distance within the bone. It is well known to derive such parameters from e.g. X-ray images.

In that situation, the image data may comprise information relating to the cortical bone of at least a part of the bone, and one of the one or more parameters may relate to a thickness, t, of the cortical bone. In addition or alternatively, one of the one or more parameters may relate to a width, w, of the cortical bone.

These thicknesses may be derived from e.g. metacarpal bones - and even from different - or both - sides thereof. However, this is not desired with all bones. If the bone is a radius, the t value or values is/are preferably determined on a radial side of the radius, and if the bone is an ulna, the t value or values is/are preferably determined on an ulnar side of the ulna.

t and w may be determined along a single line extending in a direction perpendicular to a longitudinal axis of the bone, and preferably pairs of (t,w) are determined for a plurality of lines extending in the direction and being positioned at different positions along the longitudinal direction of the bone, and at least one of the one or more parameters may be determined on the basis of pairs of (t,w) corresponding to the individual lines. In that situation, the at least one parameter may be determined on the basis of mean values of the t and w values corresponding to the individual lines.

In order to provide a better measurement, t and w values may be determined for each of more than 10, such as more than 20, such as more than 40, such as more than 60, such as more than 80, such as more than 100, such as more than 110 lines per cm. of the bone within a predetermined longitudinal part of the bone.

In another situation, at least one of the one or more parameters is derived on the basis of a determination of at least one value representing a variation of a density of cortical and/or trabecular bone of the at least part of the bone.

For example, the at least one variation value may be related to a cortical porosity of the bone.

Then, the at least one variation value may be determined by a method comprising determining, within the image data, first parts thereof relating to cortical bone and second parts positioned within the first parts, the second parts not relating to cortical bone. The pa-

rameter may then relate to e.g. a relationship between those parts, such as the relative sizes or positioning thereof.

When the image data are derived from a digitised, two-dimensional image of the at least part of the bone, the at least one variation value may be derived by determining one or more first areas in the image data relating to or representing cortical tissue and one or more second areas positioned within the first area(s), each of the second area(s) representing a local deviation in grey value. Then the local deviation in grey value may be a grey value being lower than the grey values of any surrounding areas of the first area(s), such as lower than the grey values of the surrounding areas minus a predetermined value.

When the at least one variation value is related to a cortical porosity or a variation in the cortical part of the image data, the at least one variation value may be determined by, in the image data:

- determining an outer cortical edge of the bone and denoting pixels thereon as cortical pixels,
- repeating:
  - for each, current cortical pixel, denote any of its neighbouring pixels having a grey value higher than that of itself as a cortical pixel,
  - until no non-cortical pixels in the neighbourhood of any current cortical pixel have a grey level value that is greater than the grey level value of the given current cortical pixel, thereby defining an inner cortical edge as the inner (endosteal) boundary of the final set of cortical pixels,
- denoting non-cortical image data pixels within the outer and inner cortical edges as porosity pixels, and
- deriving the at least one variation value on the basis of the cortical pixels and the porosity pixels.

Thus, a procedure is run where a single pixel on the boundary of the bone will "send out scouts" that also send out scouts in directions where the grey values increase. In that manner, areas with low grey values are identified.

Thus, the deriving step may comprise deriving the at least one variation value on the basis on a number of porosity pixels compared to a number of cortical pixels in the image data, and the deriving step may comprise deriving the at least one variation value on the basis of a number, size, and shape of groups of porosity pixels. Also, the groups of porosity pixels may be isolated groups of porosity pixels being surrounded by non-porosity pixels.

When the at least one variation value is related to a cortical porosity or a variation in the cortical part of the image data, the at least one variation value may alternatively or additionally be determined from oblong density variations in the image data, such as of parts of the image data relating to cortical tissue, such as where the at least one value is determined from oblong density variations extending at least substantially in a longitudinal direction of the bone.

These oblong variations are seen in both healthy and osteoporotic bone, and preferably, the oblong density variations have a length to width ratio of at least 1½:1, such as at least 2:1, such as at least 2½:1, preferably at least 3:1, such as at least 3.5:1, preferably at least 4:1, such as at least 4.5:1. However, for osteoporotic bones, it may be desired to actually have these longer, as investigations point to the fact that these channels in bone tend to be longer in osteoporotic bones.

The oblong density variations may have a length to width ratio in the interval 1½-8:1, such as 1.7-6:1, preferably 2-4:1. Also, it may be preferred to increase the length to width ratio due to the above-described difference between the channels in healthy and osteoporotic bones.

Normally, the oblong density variations in the image data will relate to oblong density variations, such as cavities, tunnels, channels, valleys, grooves, or the like, in the cortical bone.

Normally, the density variations of the bone have a length in the interval of 0.4-15 mm, such as in the interval 0.5-12 mm, preferably in the interval 0.8-10 mm, such as in the interval 1-8 mm, such as on the order of 2 mm, and a width in the interval 0.1-6 mm, such as in the interval 0.13-5 mm, preferably in the interval 0.15-3 mm, such as in the interval 0.17-2 mm, preferably in the interval 0.18-1 mm, such as on the order of 0.2-0.4 mm.

However, a longer length may be expected in bones diagnosed with certain types of diseases or conditions.

Also, the oblong density variations of image data may have a length in the interval of 0.4-15 mm, such as in the interval 0.5-12 mm, preferably in the interval 0.8-10 mm, such as in the interval 1-8 mm, such as on the order of 2 mm, and a width in the interval 0.1-6 mm, such as in the interval 0.13-5 mm, preferably in the interval 0.15-3 mm, such as in the interval 0.17-2 mm, preferably in the interval 0.18-1 mm, such as on the order of 0.2-0.4 mm. Naturally, this depends on the magnification in the image generating set-up. Also, slightly longer lengths may be suitable at least for bones with certain types of osteoporosis.

In one situation, the determined variation value is determined from a number of oblong density variations in the image data, a mean width thereof, and/or a mean length thereof.

Additionally or alternatively, the determined variation value may be determined from a grey value difference between the oblong density variations and other parts of the image data.

The variation value may also or alternatively be determined by:

- obtaining the power spectrum relating to the image data,
- identifying parts thereof relating to frequencies or frequency intervals corresponding to predetermined dimensions or dimension intervals of the oblong variations, and
  - a) estimating the energy at or within the frequencies or frequency intervals, and/or
  - b) removing at least substantially all other parts of the power spectrum, inversely Fourier transforming the resulting power spectrum, and determining a variation of the grey levels of the resulting image.

Additionally or alternatively, the variation value may be determined by filtering the image data with an oblong Gaussian Kernel and subsequently determining a variation of grey levels of the resulting image. In that situation, the variation value is preferably determined by filtering each two copies of the image data with an oblong Gaussian Kernel, the two oblong Gaussian kernels having different dimensions, and subsequently subtracting the two resulting image data and determining a variation of grey levels of the resulting image.

The oblong kernel(s) may have a length to width ratio of at least 1½:1, such as at least 2:1, such as at least 2½:1, preferably at least 3:1, such as at least 3.5:1, preferably at least 4:1, such as at least 4.5:1, and a length to width ratio in the interval 1½-8:1, such as 1.7-6:1, preferably 2-4:1.

Also, the or one oblong kernel may have a length in the interval of 0.4-15 mm, such as in the interval 0.5-12 mm, preferably in the interval 0.8-10 mm, such as in the interval 1-8 mm, such as on the order of 2 mm, and a width in the interval 0.1-6 mm, such as in the interval 0.13-5 mm, preferably in the interval 0.15-3 mm, such as in the interval 0.17-2 mm, preferably in the interval 0.18-1 mm, such as on the order of 0.2-0.4 mm - again, a longer kernel may be chosen for certain types of osteoporosis.

The oblong kernel may have a length in the interval of 1-15 mm, such as in the interval 1.3-10 mm, preferably in the interval 1½-6 mm, such as in the interval 2-4 mm, such as on the order of 3 mm, and a width in the interval 0.4-6 mm, such as in the interval ½-5½ mm, preferably in the interval 0.7-5 mm, such as in the interval 1-2½ mm, preferably in the interval 1.2-2 mm, such as on the order of 1½ mm - or slightly longer.

Also, the at least one variation value may determined by:

- providing a pre-determined template of the oblong density variations,
- template matching parts of the image data with the predetermined shape so as to identify oblong density variations,
- determining the variation value from:
  - the number of identified oblong density variations,
  - a grey value difference between the identified oblong density variations and other parts of the image data,
  - an average length and/or width of the identified oblong density variations.

In fact, as is also described above, different types of osteoporosis or bone disorders or defects in general are expected to generate different types of alterations of the internal structure of bones. Therefore, such a difference is contemplated to be a change of the dimensions of the oblong density variations of the cortical part of the image data. Thus, by looking for different dimensions, information may be derived as to which type of disorder or defect has actually generated the problem - and also as to which substance(s) counteract the actual problem. Thus, parameters may relate to different dimensions, or the im-

age data may be scanned with different kernel dimensions in order to determine the best fit - and thereby the actual dimensions.

In another situation, the image data comprises information relating to the trabecular structure of at least a part of a bone of the vertebrate, and the at least one variation value is derived by one or more of the following methods:

- obtaining an estimate of the parametric estimate of the power spectrum of the image data and extracting at least one of the one or more parameters as one relating to the energy distribution of the parametric estimate,
- obtaining, on the basis of image data on which a Fourier method has been used to emphasise the information in the image data relating to the trabecular structure, an estimate of a grey-level co-occurrence matrix and extracting at least one of the one or more parameters on the basis of the estimated co-occurrence matrix,
- obtaining an estimate of the projected trabecular pattern of the image data by using a Fourier method to emphasise the information in the image data relating to the trabecular structure and subjecting the manipulated image data to a morphological operation, and extracting at least one of the one or more parameters as one relating to the trabecular structure from the estimated projected trabecular pattern, and
- obtaining, on the basis of a frequency analysis of the image data, at least one of the one or more parameters as one relating to the periodicity of the trabecular structure of the part of the bone.

A vast number of different parameters may be generated in this manner. These are described in detail in WO 96/07161.

Also, the image data may comprise information relating to the trabecular structure of at least a part of a bone of the vertebrate, and wherein the at least one variation value may be derived by:

- determining values reflecting the projected trabecular density in the image data, caused by the X-ray attenuating properties of cancellous bone in the part of the bone, for each of a number of locations or areas in the image data, and
- deriving the at least one value from the variation of the determined PTD-values, preferably in the longitudinal direction of the bone.

In this manner, a density profile may be derived where from a number of parameters may be derived. This is extensively described in WO 97/42602.

Normally, when parameters are compared relating to different vertebrates or bones with different conditions, the method may include determining, from the result of the comparison, which of the one or more parameters is/are effected by the substance.

Also, it is preferred that a statistical uncertainty is derived in relation to each of the one or more parameters, and wherein the determined parameters are that/those having an effect exceeding the uncertainty(ies) relating to the parameter(s)

The effect of the substance on the bone may relate to e.g. the change in the one or more parameters or overall parameters of the bone, such as BMD, strength, bending strength, thickness, width, weight, and/or it may relate to the changes in e.g. bone formation brought about by the substance, whereby the substance may be bone forming, anti resorptive or have a heterogeneous effect. The overall effect/operation of the substance may be one relating to the function of the osteoblasts, osteocytes, osteoclasts or other parts of the vertebrae or body, such as the operation of the endocrine, paracrine or autocrine systems, such as growth hormone or growth hormone releasing substances or hormones) Nevertheless, if this operation or function has an impact on bone, this will be determinable from the image data.

Preferably, the substance is a substance known to or expected to have an effect on bones. Substances of this type are:

- an antiresorptive agent, such as an agent expected to act through a direct or indirect inhibition of the osteoclasts, such as:
  - Bisphosphonates, such as Etidronate, Clodronate, Pamidronate, Alendronate, Risedronate, Ibandronate, Zoledronate, Tiludronate, Incadronate, Neridronate, Olpadronate, EB-1053, or YH 529,
  - Hormone Replacement Therapy , such as Estrogens, Estrogens with progestogens, Tibolone, or Trimegestone,
  - Selective Estrogen Receptor Modulator (SERM), such as Raloxifene, Droloxifene, Idoxifene, Levormeloxifene, or Tamoxifen, or
  - Calcitonin, such as Salmon calcitonin, Human calcitonin, Calcitonin gene-related peptide.

- an agent with Heterogeneous Effect, such as Stanozolol, Oxandrolone, Nandrolone, Dihydrotachysterol and other vitamin D derivatives, Alfacalcidiol, Calcifero, Calcitriol, Cholecalciferol, ST-630, Calcium, Calcium with vitamin D, Ipriflavone and other isoflavones, or Strontium,
- a bone forming agent, such as an agent expected to act through a direct or indirect stimulation of the osteoblasts through a mitogenic mechanism, such as Sodium fluoride, Monofluorophosphate, Parathyroid hormone$_{1-84}$, Parathyroid hormone$_{1-34}$, Growth hormone, Growth hormone releasing compounds, IGF-1, IGF-BP3, Osteogenic potein-1, or Bone morphogenetic protein-2.

Even though virtually any vertebrate may be used, the fact that the present methods facilitate non-invasive measurement, at least the determination of which substance to administer will normally be performed on humans, as osteoporosis is rarely treated in animals. However, normally, the vertebrate may be that of a human, a horse, a large ape, a great ape, an anthropoid ape, a monkey, a pig, a cow, a rat, a rabbit, a dog, a cat.

Also, as the relevant parameters may relate to both cortical and trabecular tissue, virtually any bone in the vertebrate may be used. However, normally, the bone is taken from the group consisting of radius, femur, corpus vertebrae (L1, L2, L3, L4, L5, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, C1, C2, C3, C4, C5, C6, C7), calcaneus, talus, os carpi, metatars, metacarpi, phalanges, tibia, fibula, patella, ulna, humerus, mandible, clavicula, scapula, os coxae, os naviculare, os cuboideum, os cuneiform I, os cuneiform II, os cuneiform III, os sacrum, os coccygis.

One of the advantages of using especially image information relating to e.g. not only a single plane of the bone, as is the case when a bone has been removed, cut and the exposed structure is viewed, but rather to two-dimensional image data generated by radiation transmitted through the bone in order to obtain information relating to the structure throughout the thickness of the bone. In that situation, the image data will comprise more information, and the statistical uncertainty always incurred in measurements on organic tissue, will be reduced due to the super-positioning of the features of the bone. Also, this is more easily performed non-invasively. This also has the advantage that the bone need not be removed and destroyed in order to obtain the data. This is an advantage both seen from the patient, due to the time and resources required in order to remove and cut the

bone, and due to the fact that the same bone may be measured several times during a test.

Specifically, it has been found that a parameter such as especially cortical porosity is affected by Bisphosphonates, and especially cortical thickness is affected by HRT.

The invention also relates to a system for identifying substances for treatment of bone diseases/conditions, the system comprising:

- a data storage means comprising identifiers for a number of substances and, for each substance, data relating to parameters affected by the substance when administered to a vertebrate,
- search means for comparing one or more given parameter(s) to parameters in the data storage means in order to identify one or more substances affecting one or more given parameter(s),
- means for outputting the identity of the identified substances.

Normally, this system will be computer-based.

Thus, when presenting the system to one or more parameters, which are desired affected, the system responds by providing at least the identity of substances found to affect such parameter(s).

Preferably, the storage means are adapted to hold data relating to one or more bone parameters for each substance, each parameter relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone, and

wherein each of the one or more given parameters is one of the plurality of parameters.

The search means may be adapted to identify a combination of substances affecting the one or more given parameters of the bone.

Also, the data storage is adapted to hold data relating to at least one numerical parameter for each substance as well as information relating to whether the at least one numerical parameter is increased or decreased by the effect of the substance.

In that situation, the given parameter(s) comprise(s) at least one of the at least one numerical parameter, and wherein the search means further comprise means for receiving information relating to whether a desired effect of the substance is to reduce or increase the at least one numerical parameter.

Thus, the search means may comprise means for determining a combination of substances providing a desired effect on the bone, where the search means are adapted to take into account the information relating to the numerical parameter. This also has the advantage that, if such information is provided for a number of or all of the substances, to actually provide a specific combination of substances which take the actual parameters of an individual vertebrate into account and act to optimise the parameters of that individual. Often, the effects of a numerical parameter of a combinations of substances may simply be added or subtracted, taking into account different doses etc.

Also, and especially when a number of substances are identified, the data storage preferably further comprises, for each substance, information relating to recommendable doses to be administered. Also, the data storage may further comprise, for each substance, information relating to recommendable time periods between doses - and information relating to any known side effects of the substance.

In the following, a preferred embodiment of the invention is described in four examples and with reference to the drawing, wherein

- a test has been performed for certain substances, and wherein
- the annual changes, expressed in percentages, for the untreated group is shown in figure 1,

- the SD normalised annual changes for the untreated group is shown in figure 2,

- the annual changes, expressed in percentages, for the "HRT > 90%" group is shown in figure 3,

- the SD normalised annual changes for the "HRT > 90%" group is shown in figure 4,

- the annual changes, expressed in percentages, for bisphosphonate group is shown in figure 5,

- the SD normalised annual changes for the bisphosphonate group is shown in figure 6,

- the actual annual changes for porosity and striation for all groups are shown in figure 7,

- the annual changes, expressed in percentages, for the untreated and the bisphosphonate treated group are shown in figure 8,

- the actual annual changes for porosity and striation for the untreated and the bisphosphonate treated groups are shown in figure 9,

- the annual changes, expressed in percentages, for the untreated and the HRT treated group are shown in figure 10,

- the actual annual changes for porosity and striation for the untreated and the HRT treated group are shown in figure 11, and where

- figure 12 illustrates the result from a left hand of the porosity algorithm for metacarpal 4,3,2 and for ulna and radius,

- figure 13, illustrates the corresponding original images relating to figure 12,

- figure 14 illustrates different types of bone resorption in a cross section of a radius,

- figure 15 illustrates, in a one-dimensional case, an original profile shown together with this profile filtered with a medium frequency filter and a low frequency filter,

- figure 16 illustrates the cortical thickness t and the bone width W in a cross section of a cortical bone and the thickness profile of the bone when projected onto the film.

- figure 17 illustrates the regions used in a Hologic QDR 2000 DEXA apparatus as made by Hologic, USA,

- figure 18 illustrates the regions of interest used in the Pronosco X-posure System™ embodying the technology,

- figure 19 illustrates a system for incorporating a preferred method according to the invention.

Example 1: determination of cortical porosity in X-ray images

The actual generation of an X-ray image is well known to the skilled person. Digitising this image is also a well-known procedure. However, both procedures are described in the Applicants co-pending applications, such as WO 96/07161, WO 97/42602, and WO 99/01835.

The present method derives a value for the cortical porosity from an X-ray image comprising at least some of the metacarpals and the distal radius of a person. This part of the person makes it possible to use a low radiation dose and thereby facilitate screening of persons.

1.1 The algorithm used

The input to the algorithm is a grey scale image of a mainly cortical bone where the periosteal boundary of the bone has already been defined. The output is a segmentation of the bone into cortex pixels and non-cortex pixels, i.e. each pixel in the bone is classified as belonging either to the cortex or not. The cortex pixels of one half of the bone are a connected region. This segmentation takes place as follows:

- Rotate the bone so that it is vertical in the image
- Define all pixels on the periosteal boundary as cortex pixels
- A pixel has eight neighbours. For every cortex pixel c, investigate these eight neighbours in turn (it is not allowed to go beyond the periosteal boundary, and it is not necessary to investigate pixels that are already identified as cortex pixels. If the pixel value of the neighbour is higher than or equal to the pixel value in pixel c, add the neighbour pixel to the list of cortex pixels.
- Repeat step 3 until no further cortex pixels can be constructed.

This algorithm is called *the greedy algorithm* due to the appetite of step 4. The porosity is now defined as follows:

- At every horizontal line of the image, find the midpoint of the bone
- Move towards the cortex until the first cortex pixel is met. This defines the *cortical boundary*. (This is done in each side, however, in the radius and ulna only in one side - the radial and ulnar side, respectively). The distance to the periosteal surface is called the *cortical thickness*.
- Count the number of non-cortex pixels in the range of the cortical thickness. These are the *porosity pixels*.
- Compute the two-dimensional porosity p (small p) as the area of the porosity pixels divided by the area defined by the cortical boundary and the periosteal boundary. This is done in the bones: metacarpal 2, 3, 4, ulnar side of ulna, radial side of radius.
- Define the combination 2-d porosity as

$p(\text{combined}) = \frac{1}{2}\ (p(\text{radius}) + 1/3\ (p(\text{met1}) + p(\text{met2}) + p(\text{met3}))$

It may be desired to also have ulna take part of this calculation.

- Transform from 2-d to 3-d according to

$P = p(\text{combined})/\text{sqrt}(T/1mm) - 2$

where T is the mean metacarpal thickness

- Scale and shift P arbitrarily to lie within the range 1-9, and ensure that all future porosities are in this range by truncation to this range.

Preferably, the present calculation is performed on the "raw" image without any unsharpening or filtering - and even without a background correction removing the effect of soft tissue.

In Figure 12 is shown the result from a left hand of the porosity algorithm for metacarpal 4,3,2 and for ulna and radius. The black pixels are the porosities. Notice that endosteal "bays", which are shielded from horizontal "wind", count as porosity. This is a matter of definition and could be changed in later variants of the algorithm.

In Figure 13, the corresponding original images are illustrated.

Example 2: determination of cortical striation in X-ray images

The present methodology is preferably performed on X-ray images as those of Example 1.

In Figure 14, different types of bone resorption are illustrated in a cross section of a radius.

A preferred manner of deriving the variation value and especially for deriving information relating to spongiosation or intra-cortical resorption - cortical striation - is to derive information relating to oblong density variations in the image data.

Two manners are presently preferred in that respect, one of which uses a Difference Of Gaussians (DOG) procedure.

The Difference Of Gaussians may be seen as an equivalent to a band pass filter. In this procedure, two copies of the image data are filtered with two different filters - or Gaussian kernels having different sizes - one for each image. Naturally, this filtering will alter the image data.

In Figure 15, this is seen in a one-dimensional case, where an original profile is shown together with this profile filtered with a medium frequency filter and a low frequency filter.

Subsequently, the two images are combined, whereby differences there between are enhanced - differences relating to the frequency band between the two filters.

In two dimensions, this procedure may be used with different frequencies in the two dimensions. In this manner, oblong density variations may be identified, as different frequencies may be identified in the two directions.

Consequently, two copies of the image data are generated, one being filtered with a Gaussian kernel having a size of 4x1 mm and one having a size of 12x3 mm, the longer dimension being along the longitudinal direction of the bone - the predominant direction of the spongiosation.

When subtracting these images, information will remain as to oblong density variations having dimensions within the intervals identified by the different kernels. Subsequent to that, the spongiosation may be quantified by counting the number of such density variations, quantifying a mean grey level of the image data - over a background level - (due to the fact that only these oblong density variations are represented in the image data), or quantifying dimensions thereof.

This quantification of the density variation may be used as the variation value used in the present method.

Another method of deriving the information relating to these oblong density variations may be to Fourier transform the image data to obtain the power spectrum thereof. The image data being two dimensional, the power spectrum is also two dimensional. Therefore, points or areas may be defined at non-equal frequencies for the two dimensions, which frequencies represent oblong density variations.

The energy in such points/areas will directly relate to the number and/or "strength" (grey tone" of such density variations. This energy may itself be used to quantify the density variations, or the other parts of the power spectrum may be removed and the resulting power spectrum inversely Fourier transformed to obtain image data representing the oblong density variations. Subsequent to that, these density variations may be quantified as mentioned above in relation to the DOG.

In this respect it should be noted that investigations point to the possibility that certain types of bone diseases and conditions increase not the number of oblong channels in the bone but rather the length or dimensions thereof. Thus, by adapting the dimensions sought after using this dimension specific methodology, such variations may be both identified and quantified.

Example 3: the DXR BMD technology

In the following, a description is given of the product Pronosco X-posure System™ that incorporates the DXR BMD technique and with reference to the drawings wherein:

Rotate the bone so that it is vertical in the image.

An outer edge is found as the pixel path from the top row to the bottom row with highest average gradient or highest average curvature in the image (in the relevant side of the bone).

When the outer edge is found, the corresponding inner edge is found in at two step procedure:

Firstly, the image rows are shifted horizontally, such that the outer edge pixel in each row is aligned with (i.e. right above or right below) the edge pixels in the two neighbouring rows. The result hereof is called an aligned image.

Secondly, the aligned image is smoothed in order to reduce the disturbing effect of potential resorption spaces/cavities. The smoothing is mainly performed in the vertical direction (along the outer edge) using e.g. a standard (flat) local mean mask width dimensions 5mm times 1mm in the vertical and horizontal directions. Gaussian masks may also be applied, and the dimensions can be varied, using e.g. a mask of size 2.5mm times 0.5 mm or 10mm times 0.5 mm, depending on the image noise level and the dimensions of the given bone.

Thirdly, the inner edge is found in the smoothed aligned image as the pixel path (from the top row to the bottom row) with highest average grey level value.

Figure 16 illustrates the cortical thickness t and the bone width W. Shown is a cross section of a cortical bone and the thickness profile of the bone when projected onto the film. The projection shows a characteristic shape with local maxima at the boundaries of the medullar region (at the endosteal surface).

Figure 17 illustrates the regions used in a Hologic QDR 2000 DEXA apparatus as made by Hologic, USA. The MID region starts 15 mm below the lower edge of the radius end plate. The bottom of the MID region is defined as 10 mm above the location of the middle of the 1/3 region. The 1/3 region in turn is defined using information about the length of the forearm. See the Hologic Manual in Appendix F for a complete description of the Hologic definition of the MID.

Figure 18 illustrates the regions of interest used in the Pronosco X-posure System™ embodying the technology. Comparing with figure 2, it is seen that the ROIs in the radius and

ulna are similar - but not identical - to the regions used in the DEXA apparatus. In the metacarpals, the cortical thicknesses are determined on both sides (radial and ulnar sides), while in the radius and ulna, only the radial and ulnar sides, respectively, are determined.

Theoretical considerations concerning BMD from radiogrammetry

To define BMD in the context of e.g. a DEXA machine, one projects the bone onto a plane. In this projection, one defines a certain region or area of the bone. BMD is defined as the mineral mass of the bone projected onto this area, divided by the area itself:

BMD = Bone Mineral Mass / Area

The mass used is the ash weight, i.e. the mineral content, which is mainly constituted by Calcium. The unit of BMD is g/cm^2 or mg/cm^2.

When defining the area, care must be taken to understand the projection geometry. In the case of X-ray images and fan-beam mode of DEXA machines, there is a certain magnification *M* of the distances in the image of the bone. This magnification is dictated by the distance D_{sf} from the X-ray emitting *spot* to the *film* and the distance D_{of} from the *object* to the *film* according to the following relation:

$$M = \frac{1}{1 - D_{of} / D_{sf}}$$

This factor, M, is incorporated in the const-values used for the determination of DXR-BMD.

The X-ray images for the X-posure System™ are acquired with a nominal spot-film distance D_{sf}=100 cm. The hand rests directly on the film cassette, and this gives a distance D_{of} in the range 1-5 cm. A change of spot-film distance of 10 cm gives a change in *M* of 0.3% (assuming D_{of} = 3 cm). A change of object-film distance of 2 cm (e.g. by lifting the hand from the film) gives a change in *M* of 2%. Hence it is much more important to control the object-film distance than the spot-film distance. This is reflected in the user manual for the system.

DEXA bone densitometers utilise *absorptiometry* to estimate the BMD. These devices measure the reduction of the *intensity* of X-rays as they pass though the tissue. This is also true for Radiographic Absorptiometry (RA).

In contrast, the Pronosco X-posure System™ does not use the intensities of the image in a quantitative manner. This would require the presence of e.g. an aluminum wedge in every radiograph, as used in RA-systems.

Instead, the Pronosco X-posure System™ bases its measurement on *radiogrammetry*. Radiogrammetry is the measurement of distances on the film.

The following sections describe the radiogrammetry and the texture analysis.

Radiogrammetry and bone volume per area (VPA)

The Pronosco X-posure System™ determines the cortical bone volume using radiogrammetry.
The bone volume per area is denoted VPA. For a cylindrical bone, the following exact formula can be derived:

$$VPA = \pi * t * (1 - t / W)$$

Here W is the diameter of the bone and t is the cortical thickness, as illustrated in figure 16. If the bone is not cylindrical, the factor π is replaced by a geometrical factor f:

$$VPA = f * t * (1 - t / W)$$

The factor f depends on the shape of the bone. It can be assumed that the cross sectional shape of bones for the population covered by the BMD formula (Caucasian women) is invariant to a good approximation, i.e. that only the cortical thicknesses and the overall sizes vary, while the general *shape* is constant. With this assumption, the factor f is a constant. This assumption is eventually verified by the high correlation between DXR-BMD and DEXA-BMD demonstrated in the clinical trials.

The bone volume is used to compute the BMD estimate

$BMD = c * VPA$

This relation states that the bone volume is proportional to the bone mass. This is based on the assumption that cortical bone tissue has a constant mineral content per unit volume. This assumption is eventually verified in the high correlation between DXR-BMD and DEXA-BMD demonstrated in the clinical trials.

If, however, porosity of the bone is desired taken into account, c could be replaced by c x (1-p) where p is a measure of porosity determined from the image data. Otherwise, age may be introduced, as age and porosity in the dense bone has been found to correlate.

The measurement region for VPA

The DEXA apparatus uses the region MID shown in figure 17.

Both the DXR-BMD and the DEXA-BMD are estimates of the true BMD in the MID region. DXR-BMD arrives at this estimate using information from several bone regions. These regions are shown in figure 18.

The VPA used in DXR-BMD is computed as an average of VPAs computed in the five bones according to the formulae

$$VPA = \tfrac{1}{2} VPA_{radius/ulna} + \tfrac{1}{2} VPA_{met}$$

$$VPA_{met} = 1/3 \, (VPA_{met2} + VPA_{met3} + VPA_{met4})$$

$$VPA_{radius/ulna} = \tfrac{1}{2} (VPA_{radius} + VPA_{ulna})$$

$$VPA = \pi * t * (1 - t / W)$$

VPA is the bone volume per area and is computed from the average cortical thickness t and the outer diameter W of the bone. VPA has the dimension mm. For the metacarpals t is the average of the thicknesses in the two sides, while for radius and ulna only the outer sides are used. The axial aspects of radius and ulna protrude wing-shaped into the interosseous space to anchor the interosseous membrane.

In comparison with traditional radiogrammetry, the Pronosco X-posure System™ utilises the automation and computing power of modern PCs to compound measurements from a very large number of points along the bones to yield an average cortical thickness for a given region of a given bone. The Pronosco X-posure System™ measures the cortical thickness at 118 points per centimetre along the axis of a bone, which adds to the reproducibility and accuracy of the BMD estimate.

The use of radiogrammetry in the metacarpals to estimate the BMD in the forearm is based on the high correlation between BMD in these bones. The same applies to the use of a slightly different ROI in the radius and ulna as compared to the ROI in the predicate device. It should be noted that the ROI's in ulna and radius are positioned identically with respect to distances from the 4.0mm landmark.

In fact, presently, a correlation of 0.90 has been found between the DXR-BMD and DEXA-BMD when using the formula according to the second aspect and as described with respect to the figures.

When using the more coarse equation using merely t from the image data, a correlation of 0.88 is seen between DXR-BMD and DEXA-BMD.

Example 4: investigation of the effect of certain substances

Today bone mineral density (BMD) measurements using Dual-energy X-ray Absorptiometry (DXA) are used to assess the efficacy of anti-osteoporotic treatment with agents such as Bisphosphonates, calcitonin and HRT. It has been suggested that the changes observed in BMD during treatment with these agents only explain part of the observed reduction in fracture incidence, and that their effect also might be ascribed to the action on the bone micro architecture. A newly developed method termed Digital X-ray Radiogrammetry (DXR) may provide more detailed information about treatment effects than obtained with conventional DXA densitometry.

Objectives

To evaluate the longitudinal changes of different bone parameters measured using DXR and DXA technology in selected groups of postmenopausal women, either untreated or in treatment with hormone replacement therapy (HRT) or Bisphosphonates.

Methodology

The study was designed as open comparative, non-randomised observational study. It was planned that 120 women who had previously participated in a normative reference study with a prototype of the Pronosco X-posure System should have their bone status re-evaluated using both DXA and DXR. According to treatment status, in the period between the initial and the follow-up visit, the women where retrospectively allocated into one of the following four groups: an untreated group, a group who received HRT in more than 90% of the study period, a group who received HRT in less than 90% of the study period and a group who was treated with Bisphosphonates.

The following measurements were performed with DXA at the initial visit and at the follow-up visit: spine BMD (L2-L4), hip BMD (femoral neck) and forearm BMD (distal). For DXR the following measurements were performed at the initial visit and at the follow-up visit: DXR BMD, cortical thickness of the 2nd metacarpal (M.T. (2)), porosity and striation. DXA BMD was measured using a Norland XR-26 densitometer. DXR BMD, cortical thickness of the 2nd metacarpal, porosity and striation were measured using the Pronosco X-posure System. Porosity and striation are measured in arbitrary units on a scale from 0 to 9.

Based on the calculated mean annual changes the study was analysed with respects to changes within the groups as well as between the groups. For within group comparisons a paired t-test was used and for between group comparisons an unpaired t-test was used. For the within group comparisons SD normalised annual changes were calculated as (ΔX_{1year} / SD).

Ethics

The study was carried out in accordance with the revised Helsinki Declaration (Somerset West 1996). The local ethical committee approved the extension of the original protocol

and a new patient information and informed consent form before the study was initiated. All the participants were given written and oral information about the study before a written informed consent was obtained.

Results

The baseline demographic characteristics and the DXR BMD values for the subjects enrolled and which can be evaluated are given in table 1 illustrating demographic data and observational periods.

One hundred and twenty-six women were enrolled in the study and 113 were included in the analyses. Thirteen women were excluded due to poor quality of the radiographs taken at baseline. The main reason for discarding the radiographs was that the hand and forearm was wrongly centered. Due to the low number of women enrolled in the group who received HRT in less than 90% of the observational period no further analyses are performed on this group. In the bisphosphonate group two different agents were used for treatment. Eight women got Etidronate and the remaining 3 Alendronate.
The actual annual changes for the untreated group is shown in table 2 illiustrating actual annual changes for the untreated group (n=69).

Expressed in percentages the annual changes for the untreated group is shown in figure 1.

The SD normalised annual changes for the untreated group is shown in figure 2.

The actual annual changes for the group who received HRT in more than 90% of the observational period is shown in table 3 illustrating the actual annual changes for the "HRT>90%" group (n=28).

Expressed in percentages the annual changes for the "HRT > 90%" group is shown in figure 3.

The SD normalised annual changes for the "HRT > 90%" group is shown in figure 4.

The actual annual changes for the bisphosphonate group is shown in table 4 illustrating actual annual changes for the bisphosphonate group (n=11).

Expressed in percentages the annual changes for bisphosphonate group is shown in figure 5.

The SD normalised annual changes for the bisphosphonate group is shown in figure 6.

The actual annual changes for porosity and striation for all groups are shown in figure 7.

In order to compare the bisphosphonate treated with the untreated women of comparable age, all women from these two groups with an age between 70 and 80 years were analysed. In this age matched sub-sample a between groups comparison was made. In table 5 (illustrating the demographic data and observational periods for the subsample of untreated and bisphosphonate treated women) the demographic baseline data for the two sub-samples are shown.

When comparing the untreated with the bisphosphonate treated groups the actual annual changes in DXR BMD, cortical thickness of the 2nd metacarpal and porosity came out to be statistically significant difference. Neither spine DXA, femoral neck DXA nor distal forearm DXA were statistically significant different. The annual changes for the two groups and the p-values are given in table 6 illustrating actual annual changes for the subsample of untreated and bisphosphonate women.

Expressed in percentages the annual changes for the untreated and the bisphosphonate treated group are shown in figure 8.

The actual annual changes for porosity and striation for the untreated and the bisphosphonate treated groups are shown in figure 9. For porosity a statistically significant reduction was observed (P=0.046).

The untreated and the HRT treated group were compared with respect to the actual annual changes. The demographic data for the two groups are shown in Table 1. When comparing the untreated with the HRT treated group, with respect to the actual annual changes, cortical thickness of the 2nd metacarpal was the only parameter showing a stati-

cally significant difference. The actual annual changes for the two groups (untreated and the "HRT>90%" treated group) and the p-values are given in table 7.

Expressed in percentages the annual changes for the untreated and the HRT treated group are shown in figure 10.

The actual annual changes for porosity and striation for the untreated and the HRT treated group are shown in figure 11.

Discussion and Conclusion

In the present study the Pronosco X-posure System has for the first time been used to evaluate longitudinal changes in bones. A total of 126 women were followed for a period of two to two-and-a-half year. At baseline and at the end of the study measurements were performed with both DXA and DXR in order to compare the two methods of assessing bone status. For the evaluation of the effect of the anti-osteoporotic treatment the study participants were retrospectively divided into three groups: an untreated group, a HRT group and a bisphosphonate group.

As expected a decrease in BMD of approximately 1 to 1.5% per year was observed in the untreated group of women when measured with both DXA and DXR. The annual changes in M.T. (2), porosity and striation were also within the expected ranges when data from a cross-sectional US normative study are used as reference[1]. Looking at the SD normalised annual changes all the BMD measures and the M.T. (2) showed a statistically significant decrease.

As a result of the intervention the HRT treated group had a somewhat lower decrease in BMD compared to the untreated group. When the SD normalised annual changes were compared, hip DXA and forearm DXA showed a statistically significant decrease despite of treatment, which was in contrast to most of the parameters measured with DXR. One exception was striation, which showed a statistically significant increase. M.T. (2) was the only measure that was statistically significant difference when the untreated and HRT treated group was compared indicating an effect of estrogen on the cortical thickness.

For the bisphosphonate treated group an increases in BMD was measured at all sites expect for forearm DXA. Also for M.T. (2) a small increase was seen. Both porosity and striation showed a decrease over time. Looking at the SD normalised annual changes porosity was the only measure, which came out statistically significant. Porosity measured by DXR is believed to express the porosity of the cortical bone. Base on data from a US normative reference study it has been shown that a high level of porosity is associated with an increased fracture incidence, which emphasise the possible importance of this measure. The study results seems to indicate that treatment with Bisphosphonates have a pronounced influence on the cortical porosity. When a direct compassion was made between the untreated and the bisphosphonate treated group only DXR BMD, M.T. (2) and porosity came out statically significant different.

Results from several clinical studies where pharmacological interventions have been used seems to confirm that a simple measurement of bone mass using the DXA technology only provide part of the picture related to the changes which occurs in the bones following treatment of osteoporosis. The present clinical study seems to confirm these results by indicating that the DXR technology might be more sensitive than DXA to changes in the bones caused by anti resorptive treatment with Bisphosphonates and HRT. Further, the DXR technology might also be able to distinguish between the individual anti resorptive agents with respect to their pharmacological effects on the bones.
In conclusion, the DXR technology in comparison to DXA seems to provide a more complete picture of the processes in the bones following treatment of osteoporosis, but the result form the current observational study must be repeated in a lager population using randomised study design.

Example 5: A system for performing a preferred embodiment of the invention

In figure 19, a computer system comprising a database 1 is illustrated. The database 1 is connected to a means 2 which is used for searching in the database 1 and for outputting the results of the searches and for receiving searching criteria for use in the search.

The database 1 will comprise records each identifying a substance having an effect on bone and also information relating to one or more parameters affected by the substance. The records will also comprise information relating to doses and time intervals, which give the said effect on the bone.

Also, these parameters will be numerical parameters, whereby the information relating to a parameter will be a number relating to how much the individual parameters are affected by the doses and time intervals.

One substance may be identified by a number of records - where each record relates to different doses and/or time intervals between doses.

The means 2 comprise means for inputting parameters, which should be affected on a given bone, which has been determined to be in need of a substance. These values for these parameters are input, where after the search means identifies substances, which affect these parameters.

It may very well be that a substance will not be present which affects all the given parameters in the correct direction - or which affects all parameters to the desired degree. A substance affecting a number of the parameters may have an adverse effect on one of the given parameters - or have an effect on parameters, which are not in the given parameters - such as a parameter, which in the bone is normal. In this situation, the search means 2 will search for a combination of substances, the combined effect of which has the desired overall effect on the given - and preferably also other - parameters of the bone.

The search means 2 will finally output the information relating to the substance or substances identified. This information will relate to the identity thereof as well as information relating to doses and time intervals between doses.

Naturally, the individual elements of this system - such as the hardware and software, may be elements widely in use today, as the overall effect of the system is the identification of suitable parameters and the combination of several substances, if a single substance is not identifiable which provides the desired effect. Also, the means 1 and two may be separated, and a number of search means 2 may be physically distributed and connected to the same database 1. In fact, one and the same database 1 may provide the information required for search means 2 positioned all over the world.

	Untreated	HRT>90%	HRT<90%	Bisphos.
Enrolled subjects (n)	75	32	5	14
Evaluable subjects (n)	69	28	5	11
Observational period (days)	868	842	904	804
Age (years)*	65.6 (7.8)	63.6 (7.8)	56.6 (7.1)	75.9 (3.3)
DXR (g/cm2)*	0.498 (0.06)	0.548 (0.06)	0.560 (0.05)	0.411 (0.06)
Height (cm)*	161.8 (5.9)	163.7 (5.4)	169.1 (8.0)	155.8 (5.5)
Weight (kg)*	70.8 (15.9)	67.9 (10.8)	71.1 (8.7)	61.5 (7.1)

Table 1.

* Mean (SD)

5

	Mean	SD
DXA Spine (g/cm2)	-0.0078	0.0249
DXA Hip (g/cm2)	-0.0126	0.0199
DXA Forearm (g/cm2)	-0.0060	0.0098
DXR (g/cm2)	-0.0044	0.0068
M.T. (2) (mm)	-0.0199	0.0390
Porosity	-0.0096	0.4111
Striation	0.1113	0.4859

Table 2.

10 **Table 3.**

	Mean	SD
DXA Spine (g/cm2)	-0.0048	0.0185
DXA Hip (g/cm2)	-0.0085	0.0137
DXA Forearm (g/cm2)	-0.0036	0.0073
DXR (g/cm2)	-0.0016	0.0072
M. T. (2) (mm)	-0.0006	0.0236
Porosity	0.0145	0.5191
Striation	0.1512	0.3640

Table 4.

	Mean	SD
DXA Spine (g/cm2)	0.0154	0.0294
DXA Hip (g/cm2)	0.0018	0.0231
DXA Forearm (g/cm2)	-0.0009	0.0080
DXR (g/cm2)	0.0034	0.0076
M. T. (2) (mm)	0.0024	0.0113
Porosity	-0.3094	0.3735
Striation	-0.0903	0.4494

Table 5.

	Untreated	Bisphosphonates
N	21	10
Observational time (days)*	836 (125)	800 (144)
Age (years)*	74.3 (2.7)	76.4 (1.6)
DXR (g/cm2)*	0.453 (0.056)	0.411 (0.064)
Height (cm)*	162.7 (6.6)	155.5 (5.8)
Weight (kg)*	69.7 (13.3)	61.0 (7.3)
Menopausal age (years)*	48.9 (4.3)	48.8 (3.0)

Table 6.

	Untreated	Bisphosphonates	P-Values
N	21	10	
DXA Spine (g/cm2)*	0.0008 (0.0262)	0.0126 (0.0300)	0.287
DXA Hip (g/cm2)*	-0.0142 (0.0211)	0.0027 (0.0242)	0.063
DXA Forearm (g/cm2)*	-0.0076 (0.0092)	-0.0011 (0.0084)	0.07
DXR (g/cm2)*	-0.0037 (0.0067)	0.0038 (0.0079)	**0.01**
M. T. (2) (mm)*	-0.0154 (0.0320)	0.0011 (0.0110)	**0.043**
Porosity*	-0.0156 (0.3680)	-0.3169 (0.3928)	**0.046**
Striation*	0.1660 (0.5093)	-0.1387 (0.4423)	0.12

* Mean (SD)

Table 7.

	Untreated	HRT>90%	P-Values
N	69	28	
DXA Spine (g/cm2)*	-0.0078 (0.0249)	-0.0048 (0.0185)	0.566
DXA Hip (g/cm2)*	-0.0126 (0.0199)	-0.0085 (0.0137)	0.254
DXA Forearm (g/cm2)*	-0.0060 (0.0097)	-0.0036 (0.0073)	0.253
DXR (g/cm2)*	-0.0044 (0.0068)	-0.0016 (0.0072)	0.077
M. T. (2) (mm)*	-0.0199 (0.0390)	-0.0006 (0.0236)	**0.004**
Porosity*	0.0096 (0.4111)	0.0145 (0.5191)	0.81
Striation*	0.1113 (0.4859)	0.1512 (0.3640)	0.696

CLAIMS

1. A method of determining which substance, of a number of substances having an effect on bone, to administer to a vertebrate in need thereof, the method comprising:

- providing image data relating to one or more bones of the vertebrate,
- deriving, from the image data, one or more parameters relating to the bone,
- providing first information relating to the one or more parameters of the one or more bones of vertebrates classified as not suffering from bone diseases,
- comparing the first information and the derived one or more parameters and determining differences there between,
- providing second information relating to each of the number of substances and information relating to which of the one or more parameters of bones are affected thereby,
- determining which substance/substances to administer to the vertebrate on the basis of the result of the comparison and the second information.

2. A method according to claim 1, wherein the deriving step comprises deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

3. A method according to claim 1 or 2, the method being performed on a vertebrate or vertebrates classified as suffering from Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer or prostate cancer.

4. A method according to any of claims 1-3, wherein the determination step comprises deriving information on suitable doses or intervals of administration.

5. A method according to claim 1, wherein the second information further comprises, for each substance and for at least one numerical parameter, information relating to whether the parameter is increased or decreased by the effect of the substance.

6. A method according to claim 5, wherein the given parameter(s) comprises the at least one numerical parameter and wherein the comparing step also comprises deriving information relating to whether a desired effect of the substance is to reduce or increase the numerical parameter.

7. A method according to claim 6, wherein the determining step comprises determining a combination of substances providing the desired effect on the bone, where the determining step takes into account the information relating to the numerical parameter for each of the substances in the combination of substances.

8. A method according to any of claims 1-7, wherein the determining step comprises:

- providing, in relation to each of the one or more parameters, information relating to an uncertainty, such as a statistical uncertainty and/or an uncertainty deriving from the providing of the image data or the parameters, in the deriving of the parameter(s) and
- determining, on the basis of that uncertainty, the substance/substances only on the basis of parameter(s) significant in relation to their related uncertainties.

9. A method according to any of claims 1-8, wherein the determining step comprises determining a combination of substances, the effect of which is expected to be altering the one or more parameters toward those of the first information.

10. A method according to any of the preceding claims, wherein the step of providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby comprises:

- providing first image data relating to at least part of a bone of a vertebrate at a first point in time,
- administering one of the one or more substances to the vertebrate after the first point in time,
- providing, at a second point in time subsequent to the administering of the substance, second image data relating to the at least part of the bone,
- deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,

- comparing the one or more parameters relating to the first and second points in time, and
- providing, from the result of the comparison, information relating to which of the one or more parameters is affected by the substance.

11. A method according to claim 1, wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

12. A method according to claim 10 or 11, comprising administering the substance to the vertebrate a number of times between the first and second points in time and at predetermined doses and time intervals.

13. A method according to any of claims 10-12, the method further comprising:

- providing third image data of the at least part of the bone and at at least one third point in time between the first and second points in time,
- deriving, from the third image data, the one or more parameters, and
- comparing, in the comparing step, the one or more parameters relating to the first, second, and third points in time.

14. A method according to any of claims 10-13, wherein:

- the image data are provided relating to at least parts of bones of a number of vertebrates,
- the substance is provided to each of the vertebrates,
- the one or more parameters are derived relating to the bones of from each of the vertebrates, and
- the parameters of the vertebrates are compared in the comparing step.

15. A method according to any of claims 10-14, wherein the vertebrate or vertebrates is/are classified as suffering from Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone

diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer or prostate cancer.

16. A method according to any of the preceding claims, wherein the step of providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby comprises:

- providing a first group of vertebrates having received one of the one or more substances,
- providing a second group of vertebrates not having received the substance to any significant degree,
- providing first image data relating to at least part of the bone of each of the first group of vertebrates,
- providing second image data relating to the at least part of the bone of each of the second group of vertebrates,
- deriving, from the first image data, one or more parameters relating to the bones of the first group of vertebrates,
- deriving, from the second image data, the one or more parameters relating to the bones of the second group of vertebrates,
- comparing the one or more parameters relating to the bones of the first and second groups of vertebrates and
- providing, from the result of the comparison, information relating to which of the one or more parameters are affected by the substance,

17. A method according to claim 16, wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

18. A method according to claim 16 or 17, wherein the vertebrates of the first group of vertebrates have been classified to have or have been brought to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and wherein the vertebrates of the second group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions.

19. A method according to claim 16 or 17, wherein the vertebrates of the first and second groups of vertebrates have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions.

20. A method according to claim 19, further comprising:

- providing a third group of vertebrates, wherein the vertebrates of the third group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions,
- providing third image data relating to the bone of each of the third group of vertebrates,
- deriving, from the third image data, the one or more parameters, and (for hvert vertebrat)
- comparing, in the comparing step, the one or more parameters of the first, second, and third groups of vertebrates.

21. A method according to claim 18 or 19 wherein the disease or condition or group of diseases or conditions is/are chosen from: Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer of prostate cancer.

22. A method according to any of claims 16-21, comprising providing the first, second and optionally third image data at at least two different points in time between which the first group has received at least part of the substance received, and wherein the comparing step comprises comparing the parameters derived from the image data obtained at the at least two points in time.

23. A method according to claim any of the preceding claims, wherein the step of providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby comprises:

- providing first image data relating to at least part of a bone of a vertebrate at a first point in time,
- administering one of the one or more substances to the vertebrate after the first point in time,
- providing, at a second point in time subsequent to the providing of the substance, second image data relating to the at least part of the bone,
- deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,
- comparing the one or more parameters relating to the first and second points in time, and
- determining, from the result of the comparison, whether the substance has any effect on the bone, and
- providing, if the substance has an effect on the bone, information that the substance has an effect on the bone and which of the one or more parameters is affected thereby.

24. A method according to claim 23, wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

25. A method according to claim 23 or 24, further comprising administering the substance to the vertebrate a number of times between the first and second points in time and at predetermined doses and time intervals.

26. A method according to claim 23, 24, or 25, the method further comprising:

- providing third image data of the at least part of the bone and at at least one third point in time between the first and second points in time,
- deriving, from the third image data, the one or more parameters, and
- comparing, in the comparing step, the one or more parameters relating to the first, second, and third points in time.

27 A method according to any of claims 23-26, wherein:

- the image data are provided of at least parts of bones of a number of vertebrates,
- the substance is provided to each of the number of vertebrates,
- the one or more parameters are derived from each of the number of vertebrates, and
- the parameters of the vertebrates are compared in the comparing step.

28. A method according to any of the preceding claims, wherein the vertebrate or vertebrates is/are classified as suffering from Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer or prostate cancer.

29. A method according to any of the claims, wherein the step of providing second information relating to one or more substances affecting bones and information relating to which of the one or more parameters of bones are affected thereby comprises:

- providing a first group of vertebrates having received one of the one or more substances,
- providing a second group of vertebrates not having received the substance to any significant degree,
- providing first image data relating to at least part of the bone of each vertebrate of the first group of vertebrates,
- providing second image data relating to the at least part of the bone of each vertebrate of the second group of vertebrates,
- deriving, from the first image data, one or more parameters relating to the bones of the first group of vertebrates,
- deriving, from the second image data, the one or more parameters relating to the bones of the second group of vertebrates,
- comparing the one or more parameters relating to the first and second groups of vertebrates,
- determining, from the result of the comparison, whether the substance has any effect on the bones of the vertebrates of the first group, and

- providing, if the substance has an effect on the bone, information that the substance has an effect on the bone and which of the one or more parameters is affected thereby.

30. A method according to claim 29, wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

31. A method according to claim 29 or 30, wherein the vertebrates of the first group of vertebrates have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and wherein the vertebrates of the second group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions.

32. A method according to claim 29, wherein the vertebrates of the first and second groups of vertebrates have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions.

33. A method according to claim 32, further comprising:

- providing a third group of vertebrates, wherein the vertebrates of the third group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions,
- providing third image data relating to the bone of each of the third group of vertebrates,
- deriving, from the third image data, the one or more parameters, and
- comparing, in the comparing step, the one or more parameters of the first, second, and third groups of vertebrates.

34. A method according to claim 31 or 32, wherein the disease or condition or group of diseases or conditions is/are chosen from Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone

diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer or prostate cancer.

35. A method according to any of claims 29-34, further comprising providing the first, second and optionally third sets of image data at at least two different points in time between which the first group has received at least part of the substance received, and wherein the comparing step comprises comparing the image data obtained at the at least two points in time.

36. A method of differentiating between different types of bone lesions/defects/conditions, the method comprising:

a) providing image data on a bone in at least one vertebrate, which is suspected to suffer from a first bone disease or condition,

b) providing image data on a bone in at least one vertebrate, which is suspected to suffer from a second bone disease or condition

c) deriving one or more parameters relating to the bones based on said image data provided in step a) and b),

d) providing information relating to said one or more parameters of bones of vertebrates not suffering from the bone disease or condition,

e) comparing the one or more parameters obtained in step b) with the one or more parameters obtained in step c) to determine differences in said parameters, and

f) identifying, on the basis of the information provided in step d) and the result of the comparison of step e), one or more parameters relating to said bones of said vertebrates and being correlated to one or each of the first and second bone diseases or conditions.

37. A method according to claim 36, wherein the deriving step comprises deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

38. A method of determining the effect of a substance on a bone of a vertebrate, the method comprising:

- providing first image data relating to at least part of the bone at a first point in time,
- administering the substance to the vertebrate after the first point in time,

- providing, at a second point in time subsequent to the administering of the substance, second image data relating to the at least part of the bone,
- deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,
- comparing the one or more parameters relating to the first and second points in time, and
- determining, from the result of the comparison, the effect of the substance on the bone,

wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

39. A method according to claim 38, comprising administering the substance to the vertebrate a number of times between the first and second points in time and at predetermined doses and time intervals.

40. A method according to claim 38 or 39, the method further comprising:

- providing third image data of the at least part of the bone and at at least one third point in time between the first and second points in time,
- deriving, from the third image data, the one or more parameters, and
- comparing, in the comparing step, the one or more parameters relating to the first, second, and third points in time.

41. A method according to any of claims 38-40, wherein:

- the image data are provided relating to at least parts of bones of a number of vertebrates,
- the substance is provided to each of the vertebrates,
- the one or more parameters are derived from each of the vertebrates, and
- the parameters of the vertebrates are compared in the comparing step

in order to provide information relating to an overall effect of the substance on bone statistical analysis of the parameters of the vertebrates.

42. A method according to any of claims 38-41, wherein the vertebrate or vertebrates is/are classified as suffering from Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer or prostate cancer.

43. A method of determining the effect of a substance on a bone of a vertebrate, the method comprising:

- providing a first group of vertebrates having received the substance,
- providing a second group of vertebrates not having received the substance to any significant degree,
- providing first image data relating to at least part of the bone of each of the first group of vertebrates,
- providing second image data relating to the at least part of the bone of each of the second group of vertebrates,
- deriving, from the first image data, one or more parameters relating to the bones of the first group of vertebrates,
- deriving, from the second image data, the one or more parameters relating to the bones of the second group of vertebrates,
- comparing the one or more parameters relating to the bones of the first and second groups of vertebrates, and
- determining, from the result of the comparison, the effect of the substance on the bones of the vertebrates of the first group,

wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

44. A method according to claim 43, wherein the vertebrates of the first group of vertebrates have been classified to have or have been brought to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and wherein the vertebrates of the second group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions.

45. A method according to claim 43, wherein the vertebrates of the first and second groups of vertebrates have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions.

46. A method according to claim 45, further comprising:

- providing a third group of vertebrates, and wherein the vertebrates of the third group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions,
- providing third image data relating to the bone of each of the third group of vertebrates,
- deriving, from the third image data, the one or more parameters, and
- comparing, in the comparing step, the one or more parameters of the first, second, and third groups of vertebrates.

47. A method according to claim 44 or 45 wherein the disease or condition or group of diseases or conditions is/are chosen from: Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer of prostate cancer.

48. A method according to any of claims 43-47, comprising providing the first, second and optionally third image data at at least two different points in time between which the first group has received at least part of the substance received, and wherein the comparing step comprises comparing the parameters derived from the image data obtained at the at least two points in time.

49. A method of screening a test substance to identify whether the substance has an effect on a bone of a vertebrate, the method comprising:

- providing first image data relating to at least part of the bone at a first point in time,
- administering the substance to the vertebrate after the first point in time,
- providing, at a second point in time subsequent to the providing of the drug, second image data relating to the at least part of the bone,
- deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,
- comparing the one or more parameters relating to the first and second points in time, and
- determining, from the result of the comparison, whether the substance has any effect on the bone,

wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

50. A method according to claim 49, further comprising administering the substance to the vertebrate a number of times between the first and second points in time and at predetermined doses and time intervals.

51. A method according to claim 49 or 50, the method further comprising providing third image data of the at least part of the bone and at at least one third point in time between the first and second points in time, deriving, from the third image data, the one or more parameters, and comparing, in the comparing step, the one or more parameters relating to the first, second, and third points in time.

52. A method according to any of claims 49-51, wherein the image data are provided of at least parts of bones of a number of vertebrates, where the substance is provided to the number of vertebrates, where the one or more parameters are derived from each of the vertebrates, and wherein the parameters of the vertebrates are compared in the

comparing step in order to provide information relating to an overall effect of the substance on bone.

53. A method according to any of claims 49-52, wherein the vertebrate or vertebrates is/are classified as suffering from Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer or prostate cancer.

54. A method of screening a test substance to identify whether the substance has an effect on a bone of a vertebrate, the method comprising:

- providing a first group of vertebrates having received the substance,
- providing a second group of vertebrates not having received the substance to any significant degree,
- providing first image data relating to at least part of the bone of each vertebrate of the first group of vertebrates,
- providing second image data relating to the at least part of the bone of each vertebrate of the second group of vertebrates,
- deriving, from the first image data, one or more parameters relating to the bones of the first group of vertebrates,
- deriving, from the second image data, the one or more parameters relating to the bones of the second group of vertebrates,
- comparing the one or more parameters relating to the first and second groups of vertebrates and
- determining, from the result of the comparison, whether the substance has any effect on the bones of the vertebrates of the first group

wherein the deriving steps comprise deriving, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone.

55. A method according to claim 54, wherein the vertebrates of the first group of vertebrates have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions, and wherein the vertebrates of the second group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions.

56. A method according to claim 54, wherein the vertebrates of the first and second groups of vertebrates have been classified to have a given disease or condition or a disease or condition within a given group of diseases or conditions.

57. A method according to claim 56, further comprising:

- providing a third group of vertebrates, and wherein the vertebrates of the third group of vertebrates have been classified to not have the given disease or condition or a disease or condition within the given group of diseases or conditions,
- providing third image data relating to the bone of each of the third group of vertebrates,
- deriving, from the third image data, the one or more parameters, and
- comparing, in the comparing step, the one or more parameters of the first, second, and third groups of vertebrates.

58. A method according to claim 55 or 56 wherein the disease or condition or group of diseases or conditions is/are chosen from Bone disorders or conditions, such as type I and II osteoporosis, glucocroticoid induced osteoporosis and other types of drug induced osteoporosis, osteomalacia, hyper- and hypoparathyroidism, hyper- and hypothyroidism, diabetes (IDDM), hypopituitarism, osteoarthritis, rheumatoid arthritis, genetic bone diseases such as osteogenesis imperfecta, Paget's bone disease, myeloma or cancer, such as breast cancer or prostate cancer.

59. A method according to any of claims 54-58, further comprising providing the first, second and optionally third sets of image data at at least two different points in time between which the first group has received at least part of the substance received, and wherein the comparing step comprises comparing the image data obtained at the at least two points in time.

60. A method according to any of the preceding claims, wherein each vertebrate is a laboratory or test animal, and wherein the method comprises finally sacrificing the animal.

61. A method according to any of the preceding claims, wherein the image data are obtained by a non-invasive method, such as by exposing the bone or bones to radiation, such as X-rays, performing a CT scanning, a NMR scanning or exposing the bone or bones to ultra sound.

62. A method according to claim 61, wherein the image data are two-dimensional image data relating to at least part of the bone or bones.

63. A method according to any of the preceding claims, wherein the image data comprise information relating to the cortical bone of at least a part of the bone, and wherein one of the one or more parameters relates to a thickness, t, of the cortical bone.

64. A method according to any of the preceding claims, wherein the image data comprise information relating to the cortical bone of at least a part of the bone, and wherein one of the one or more parameters relates to a width, w, of the bone.

65. A method according to claim 63, wherein the bone is a radius, and wherein the t value or values is/are determined on a radial side of the radius.

66. A method according to claim 63, wherein the bone is an ulna, and wherein the t value or values is/are determined on an ulnar side of the ulna.

67. A method according to claims 63 and 64, wherein t and w are determined along a single line extending in a direction perpendicular to the bone.

68. A method according to claim 67, wherein pairs of (t,w) are determined for a plurality of lines extending in the direction perpendicular to the bone and being positioned at different positions along the longitudinal direction of the bone, and wherein at least one of the one or more parameters is determined on the basis of pairs of (t,w) corresponding to the individual lines.

69. A method according to claim 68, wherein the at least one parameter is determined on the basis of mean values of the t and w values corresponding to the individual lines.

70. A method according to claim 68 or 69, wherein t and w values are determined for each of more than 10, such as more than 20, such as more than 40, such as more than 60, such as more than 80, such as more than 100, such as more than 110 lines per cm. of the bone within a predetermined longitudinal part of the bone.

71. A method according to any of the preceding claims, wherein one or more parameters relate to a variation value and wherein at least one variation value is related to a cortical porosity of the bone.

72. A method according to any of the preceding claims, wherein one or more parameters relate to a variation value and the at least one variation value is determined by a method comprising:

- determining, within the image data, first parts thereof relating to cortical bone and second parts positioned within the first parts, the second parts not relating to cortical bone.

73. A method according to any of the preceding claims, wherein the image data are derived from a digitised, two-dimensional image of the at least part of the bone, and wherein one or more parameters relate to a variation value and at least one variation value is derived by determining one or more first areas in the image data relating to or representing cortical tissue and one or more second areas positioned within the first area(s), each of the second area(s) representing a local deviation in grey value.

74. A method according to claim 73, wherein the local deviation in grey value is a grey value being lower than the grey values of any surrounding areas of the first area(s), such as lower than the grey values of the surrounding areas minus a predetermined value.

75. A method according to any of the preceding claims, wherein one or more parameters relate to a variation value and at least one variation value is determined by, in the image data:

- determining an outer cortical edge of the bone and denoting pixels thereon as cortical pixels,

- repeating:
  - for each current, cortical pixel, denote any of its neighbouring pixels having a grey value higher than that of itself as a cortical pixel,
  - until no non-cortical pixels in the neighbourhood of any current cortical pixel have a grey level value that is greater than the grey level value of the given current cortical pixel, thereby defining an inner cortical edge as the inner (endosteal) boundary of the final set of cortical pixels,
- denoting non-cortical image data pixels within the outer and inner cortical edges as porosity pixels, and
- deriving the at least one variation value on the basis of the cortical pixels and the porosity pixels.

76. A method according to claim 75, wherein the deriving step comprises deriving the at least one variation value on the basis on a number of porosity pixels compared to a number of cortical pixels in the image data,

77. A method according to claim 75, wherein the deriving step comprises deriving the at least one variation value on the basis of a number, size and/or shape of groups of porosity pixels.

78. A method according to claim 77, wherein the groups of porosity pixels are isolated groups of porosity pixels being surrounded by cortical pixels.

79. A method according to any of the preceding claims, wherein one or more parameters relate to a variation value and at least one variation value is determined from oblong density variations in the image data, such as of parts of the image data relating to cortical tissue.

80. A method according to claim 79, wherein the at least one value is determined from oblong density variations extending at least substantially in a longitudinal direction of the bone.

81. A method according to claim 79 or 80, wherein the oblong density variations have a length to width ratio of at least 1½:1, such as at least 2:1, such as at least 2½:1,

preferably at least 3:1, such as at least 3.5:1, preferably at least 4:1, such as at least 4.5:1.

82. A method according to any of claims 79-81, wherein the oblong density variations have a length to width ratio in the interval 1½-8:1, such as 1.7-6:1, preferably 2-4:1.

83. A method according to any of claims 79-82, wherein the oblong density variations in the image data relate to oblong density variations, such as cavities, tunnels, channels, valleys, grooves, or the like, in the cortical bone.

84. A method according to claim 83, wherein the density variations of the bone have a length in the interval of 0.4-15 mm, such as in the interval 0.5-12 mm, preferably in the interval 0.8-10 mm, such as in the interval 1-8 mm, such as on the order of 2 mm, and a width in the interval 0.1-6 mm, such as in the interval 0.13-5 mm, preferably in the interval 0.15-3 mm, such as in the interval 0.17-2 mm, preferably in the interval 0.18-1 mm, such as on the order of 0.2-0.4 mm.

85. A method according to any of claims 79-84, wherein the oblong density variations of image data have a length in the interval of 0.4-15 mm, such as in the interval 0.5-12 mm, preferably in the interval 0.8-10 mm, such as in the interval 1-8 mm, such as on the order of 2 mm, and a width in the interval 0.1-6 mm, such as in the interval 0.13-5 mm, preferably in the interval 0.15-3 mm, such as in the interval 0.17-2 mm, preferably in the interval 0.18-1 mm, such as on the order of 0.2-0.4 mm.

86. A method according to any of claims 79-85, wherein the determined variation value is determined from a number of oblong density variations in the image data, a mean width thereof, and/or a mean length thereof.

87. A method according to any of claims 79-86, wherein the determined variation value is determined from a grey value difference between the oblong density variations and other parts of the image data.

88. A method according to any of claims 79-87, wherein the variation value is determined by:

- obtaining a power spectrum relating to the image data,

- identifying parts thereof relating to frequencies or frequency intervals corresponding to predetermined dimensions or dimension intervals of the oblong variations by:
  - a) estimating an energy at or within the frequencies or frequency intervals, and/or
  - b) removing at least substantially all other parts of the power spectrum, and
- inversely Fourier transforming the resulting power spectrum, and
- determining a variation of the grey levels of the resulting image.

89. A method according to any of claims 79-87, wherein the variation value is determined by filtering the image data with an oblong Gaussian Kernel and subsequently determining a variation of grey levels of the resulting image.

90. A method according to claim 89, wherein the variation value is determined by filtering each of the image data and the resulting image with an oblong Gaussian Kernel, the two oblong Gaussian kernels having different dimensions, and subsequently subtracting the two resulting image data and determining a variation of grey levels of the resulting image.

91. A method according to claim 89 or 90, wherein the oblong kernel(s) has/have a length to width ratio of at least 1½:1, such as at least 2:1, such as at least 2½:1, preferably at least 3:1, such as at least 3.5:1, preferably at least 4:1, such as at least 4.5:1.

92. A method according to any of claims 89-91, wherein the oblong kernel(s) has/have a length to width ratio in the interval 1½-8:1, such as 1.7-6:1, preferably 2-4:1.

93. A method according to any of claims 71-92, wherein the or one oblong kernel has a length in the interval of 0.4-15 mm, such as in the interval 0.5-12 mm, preferably in the interval 0.8-10 mm, such as in the interval 1-8 mm, such as on the order of 2 mm, and a width in the interval 0.1-6 mm, such as in the interval 0.13-5 mm, preferably in the interval 0.15-3 mm, such as in the interval 0.17-2 mm, preferably in the interval 0.18-1 mm, such as on the order of 0.2-0.4 mm.

94. A method according to claim 90, wherein one oblong kernel has a length in the interval of 1-15 mm, such as in the interval 1.3-10 mm, preferably in the interval 1½-6 mm,

such as in the interval 2-4 mm, such as on the order of 3 mm, and a width in the interval 0.4-6 mm, such as in the interval ½-5½ mm, preferably in the interval 0.7-5 mm, such as in the interval 1-2½ mm, preferably in the interval 1.2-2 mm, such as on the order of 1½ mm.

95. A method according to any of claims 79-94, wherein the at least one variation value is determined by:

- providing a pre-determined template of the oblong density variations,
- template matching parts of the image data with the predetermined shape so as to identify oblong density variations,
- determining the variation value from:
  - the number of identified oblong density variations,
  - a grey value difference between the identified oblong density variations and other parts of the image data, or
  - an average length and/or width of the identified oblong density variations.

96. A method according to any of the preceding claims, wherein the image data comprising information relating to the trabecular structure of at least a part of a bone of the vertebrate, and wherein one or more parameters relate to a variation value and at least one variation value is derived by one or more of the following methods:

- obtaining an estimate of the parametric estimate of the power spectrum of the image data and extracting at least one of the one or more parameters as one relating to the energy distribution of the parametric estimate,
- obtaining, on the basis of image data on which a Fourier method has been used to emphasise the information in the image data relating to the trabecular structure, an estimate of a grey-level co-occurrence matrix and extracting at least one of the one or more parameters on the basis of the estimated co-occurrence matrix,
- obtaining an estimate of the projected trabecular pattern of the image data by using a Fourier method to emphasise the information in the image data relating to the trabecular structure and subjecting the manipulated image data to a morphological operation, and extracting at least one of the one or more parameters as one relating to the trabecular structure from the estimated projected trabecular pattern, and

- obtaining, on the basis of a frequency analysis of the image data, at least one of the one or more parameters as one relating to the periodicity of the trabecular structure of the part of the bone.

97. A method according to any of the preceding claims, wherein the image data comprising information relating to the trabecular structure of at least a part of a bone of the vertebrate, and wherein one or more parameters relate to a variation value and at least one variation value is derived by:

- determining values reflecting the projected trabecular density in the image data, caused by the X-ray attenuating properties of cancellous bone in the part of the bone, for each of a number of locations or areas in the image data, and
- deriving the at least one value from the variation of the determined PTD-values, preferably in the longitudinal direction of the bone.

98. A method according to any of the preceding claims, further including determining, from the result of the comparison, which of the one or more parameters is/are effected by the substance.

99. A method according to claim 98, wherein a statistical uncertainty is derived in relation to each of the one or more parameters, and wherein the determined parameter(s) is/are that/those having an effect exceeding the uncertainty(ies) relating to the parameter(s)

100. A method according to any of the preceding claims, wherein the substance is a substance known to or expected to have an effect on bones.

101. A method according to claim 100, wherein the substance is known to or expected to be:

- an antiresorptive agent, such as an agent expected to act through a direct or indirect inhibition of the osteoclasts, such as:
  - Bisphosphonates, such as Etidronate, Clodronate, Pamidronate, Alendronate, Risedronate, Ibandronate, Zoledronate, Tiludronate, Incadronate, Neridronate, Olpadronate, EB-1053, or YH 529,
  - Hormone Replacement Therapy, such as Estrogens, Estrogens with progestogens, Tibolone, or Trimegestone,

- Selective Estrogen Receptor Modulator (SERM), such as Raloxifene, Droloxifene, Idoxifene, Levormeloxifene, or Tamoxifen, or
- Calcitonin, such as Salmon calcitonin, Human calcitonin, Calcitonin gene-related peptide,
- an agent with Heterogeneous Effect, such as Stanozolol, Oxandrolone, Nandrolone, Dihydrotachysterol and other vitamin D derivatives, Alfacalcidiol, Calcifero, Calcitriol, Cholecalciferol, ST-630, Calcium, Calcium with vitamin D, Ipriflavone and other isoflavones, or Strontium,
- a bone forming agent, such as an agent expected to act through a direct or indirect stimulation of the osteoblasts through a mitogenic mechanism, such as Sodium fluoride, Monofluorophosphate, Parathyroid hormone$_{1-84}$, Parathyroid hormone$_{1-34}$, Growth hormone, Growth hormone releasing compounds, IGF-1, IGF-BP3, Osteogenic protein-1, or Bone morphogenetic protein-2.

102. A method according to any of the preceding claims, wherein the vertebrate is a human, a horse, a large ape, a great ape, an anthropoid ape, a pig, a cow, a rat, a rabbit, a dog, a cat.

103. A method according to any of the preceding claims, wherein the bone is taken from the group consisting of radius, femur, corpus vertebrae (L1, L2, L3, L4, L5, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, C1, C2, C3, C4, C5, C6, C7), calcaneus, talus, os carpi, metatars, metacarpi, phalanges, tibia, fibula, patella, ulna, humerus, mandible, clavicula, scapula, os coxae, os naviculare, os cuboideum, os cuneiform I, os cuneiform II, os cuneiform III, os sacrum, os coccygis.

104. A method according to any of the preceding claims, wherein the image data relate to information relating to bone tissue through a thickness of the bone.

105. A system for identifying one or more substances for treatment of bone diseases/conditions, the system comprising:

- a data storage means for holding identifiers for a number of substances and, for each substance, data relating to one or more bone parameters affected by the substance when administered to a vertebrate,

- search means for comparing one or more given bone parameters to parameters in the data storage means in order to identify one or more substances affecting the one or more given parameters,
- means for outputting information relating to the identified substance(s).

106. A system according to claim 105, wherein the storage means are adapted to hold data relating to one or more bone parameters for each substance, each parameter relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone, and

wherein each of the one or more given parameters is one of the plurality of parameters.

107. A system according to claim 105 or 106, wherein the search means are adapted to identify a combination of substances affecting the one or more given parameters of the bone.

108. A system according to any of claims 105-107, wherein the data storage is adapted to hold data relating to at least one numerical parameter for each substance as well as information relating to whether the at least one numerical parameter is increased or decreased by the effect of the substance.

109. A system according to claim 108, wherein the given parameter(s) comprise(s) at least one of the at least one numerical parameter, and wherein the search means further comprise means for receiving information relating to whether a desired effect of the substance is to reduce or increase the at least one numerical parameter.

110. A system according to claim 108 or 109, wherein the search means comprise means for determining a combination of substances providing a desired effect on the bone, where the search means are adapted to take into account the information relating to the numerical parameter.

111. A system according to any of claims 105-110, wherein the data storage further comprises, for each substance, information relating to one or more recommendable doses to be administered to a person.

112. A system according to any of claims 105-111, wherein the data storage further comprises, for each substance, information relating to recommendable time periods between doses to be administered to a person.

113. A system according to any of claims 105-112, wherein the data storage further comprises, for each substance, information relating to any known side effects of the substance when administered to a person.

114. An apparatus for providing information relating to the effect of a substance on a vertebrate, the apparatus comprising:

- means for providing image data relating to one or more bones of the vertebrate,
- means for deriving, from the image data, one or more parameters relating to the bone,
- means for providing first information relating to the one or more parameters of the one or more bones of vertebrates classified as not suffering from bone diseases,
- means for comparing the first information and the derived one or more parameters and determining differences there between,
- means for providing second information relating to each of the number of substances and information relating to which of the one or more parameters of bones are affected thereby,
- means for determining which substance/substances to administer to the vertebrate on the basis of the result of the comparison and the second information.

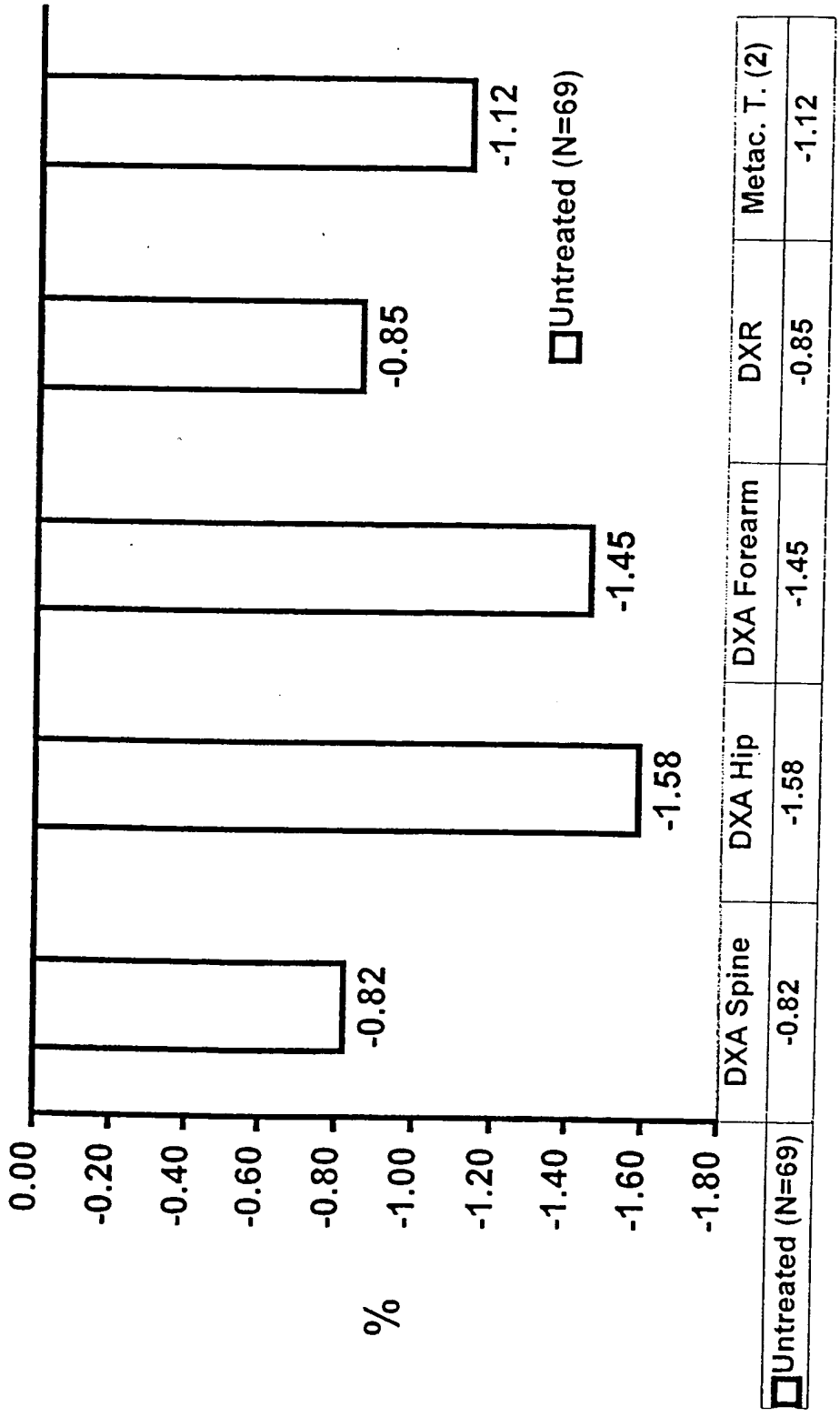
115. An apparatus for determining the effect of a substance on a bone of a vertebrate, the apparatus comprising:

- means for providing first image data relating to at least part of the bone at a first point in time, (preferably - prior to)
- means for providing, at a second point in time, second image data relating to the at least part of the bone, (preferably - subsequent)
- means for deriving, from the first image data, one or more parameters relating to the bone at the first point in time,
- means for deriving, from the second image data, the one or more parameters relating to the bone at the second point in time,

the deriving means being adapted to derive, from the image data, one or more parameters relating to:

- one or more physical distances in the bone or the image data relating to the bone and/or
- a variation of a density of cortical and/or trabecular bone of the bone

- means for comparing the one or more parameters relating to the first and second points in time, and
- means for determining, from the result of the comparison, the effect of the substance on the bone.



Annual changes expressed in percentages for the untreated (n=69)

Fig. 1

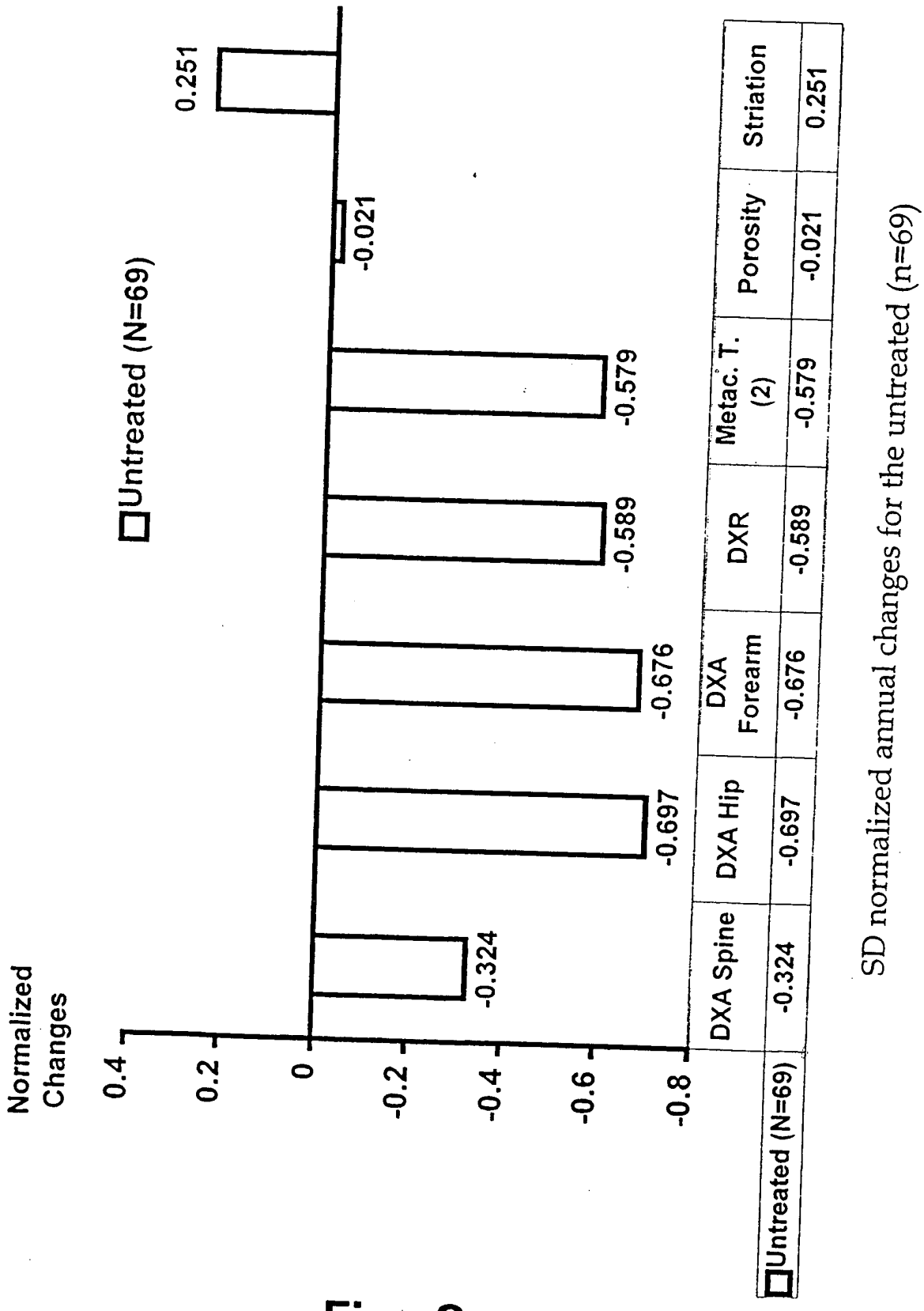
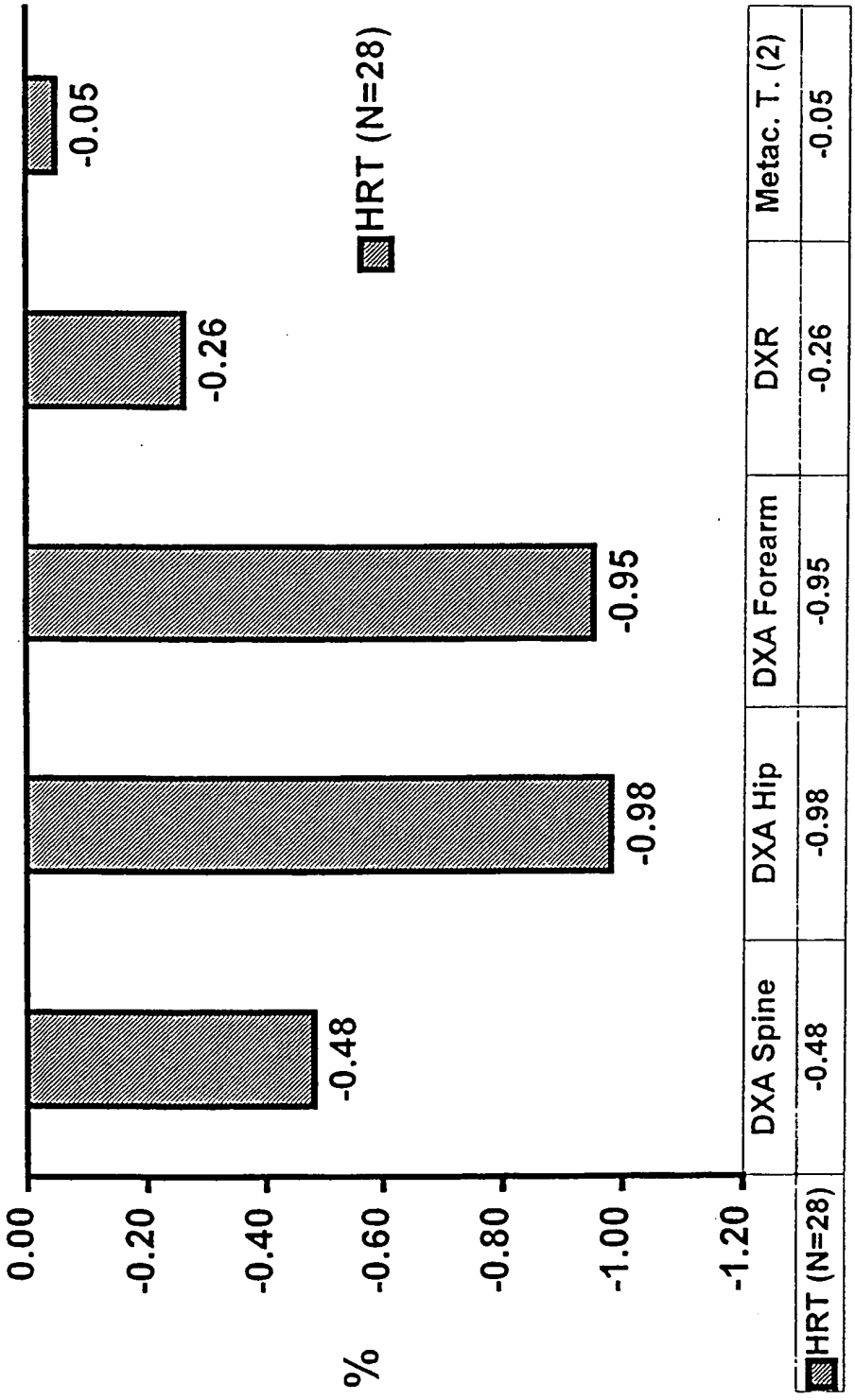
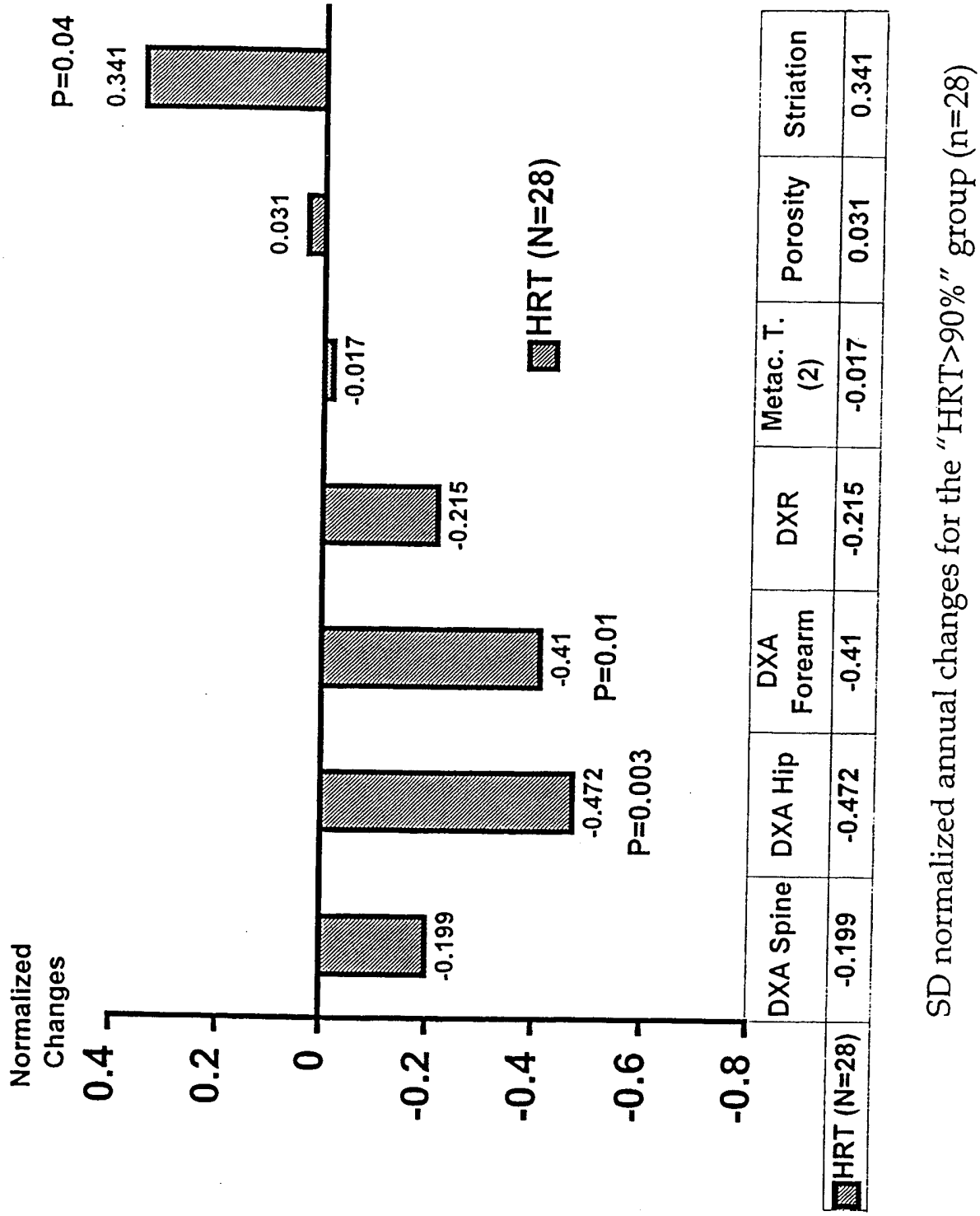

Normalized Changes
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
Untreated (N=69)
0.251
-0.021
-0.579
-0.589
-0.676
-0.697
-0.324
DXA Spine
DXA Hip
DXA Forearm
DXR
Metac. T. (2)
Porosity
Striation
Untreated (N=69)
-0.324
-0.697
-0.676
-0.589
-0.579
-0.021
0.251
SD normalized annual changes for the untreated (n=69)

Fig. 2



Annual changes expressed in percentages for the "HRT > 90%" group

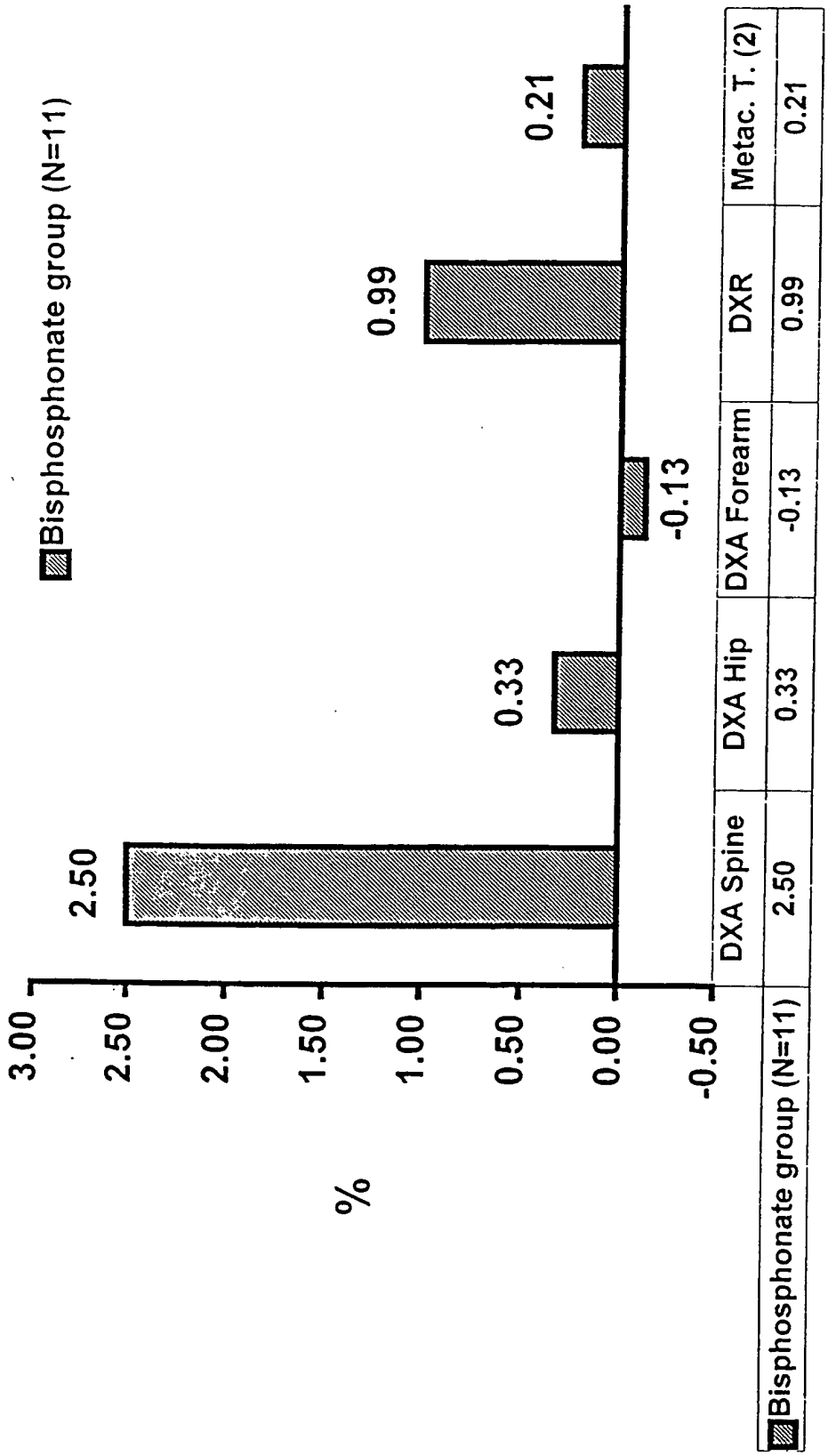
Fig. 3



SD normalized annual changes for the "HRT>90%" group (n=28)

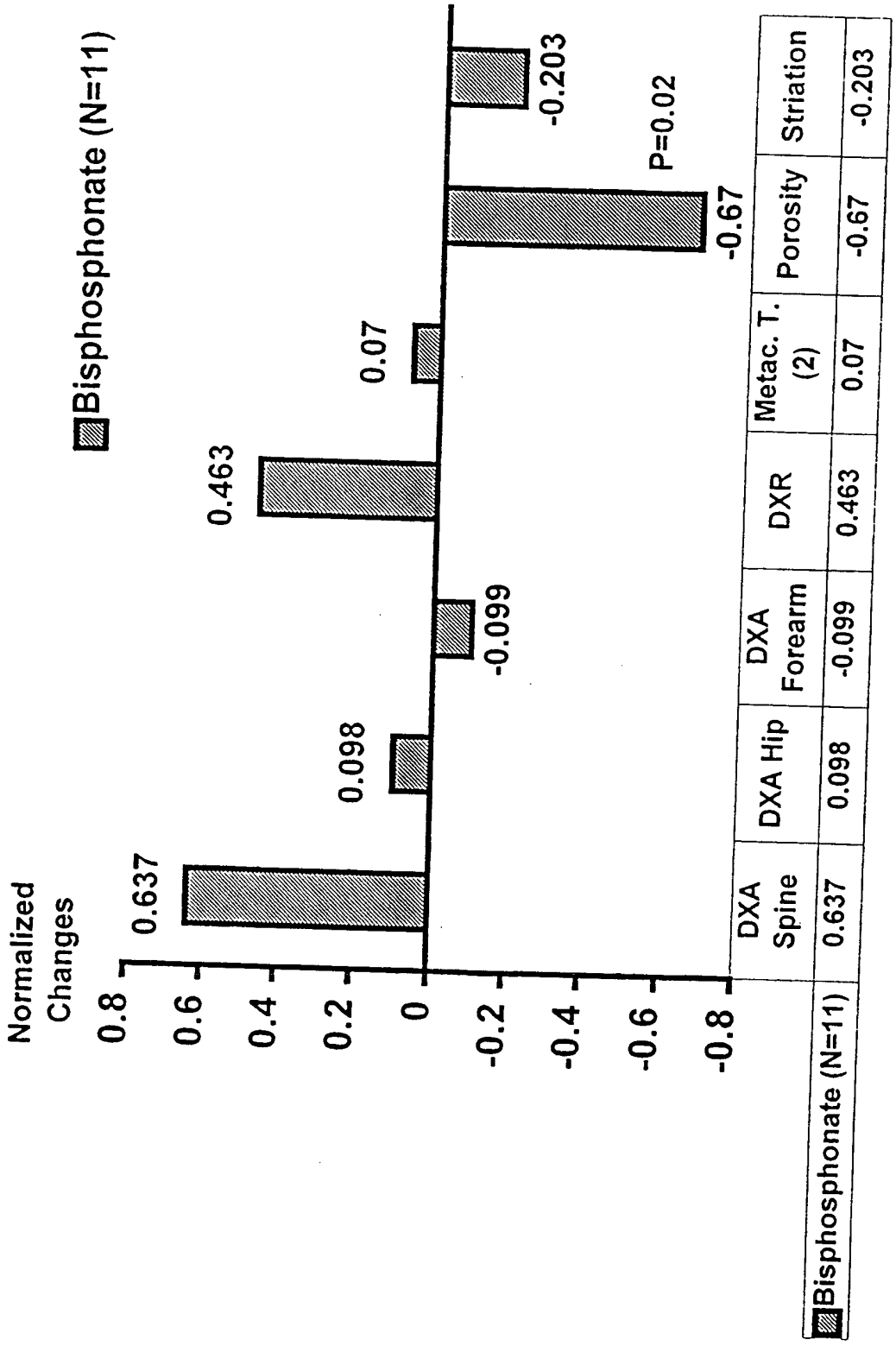
Fig. 4

5/21



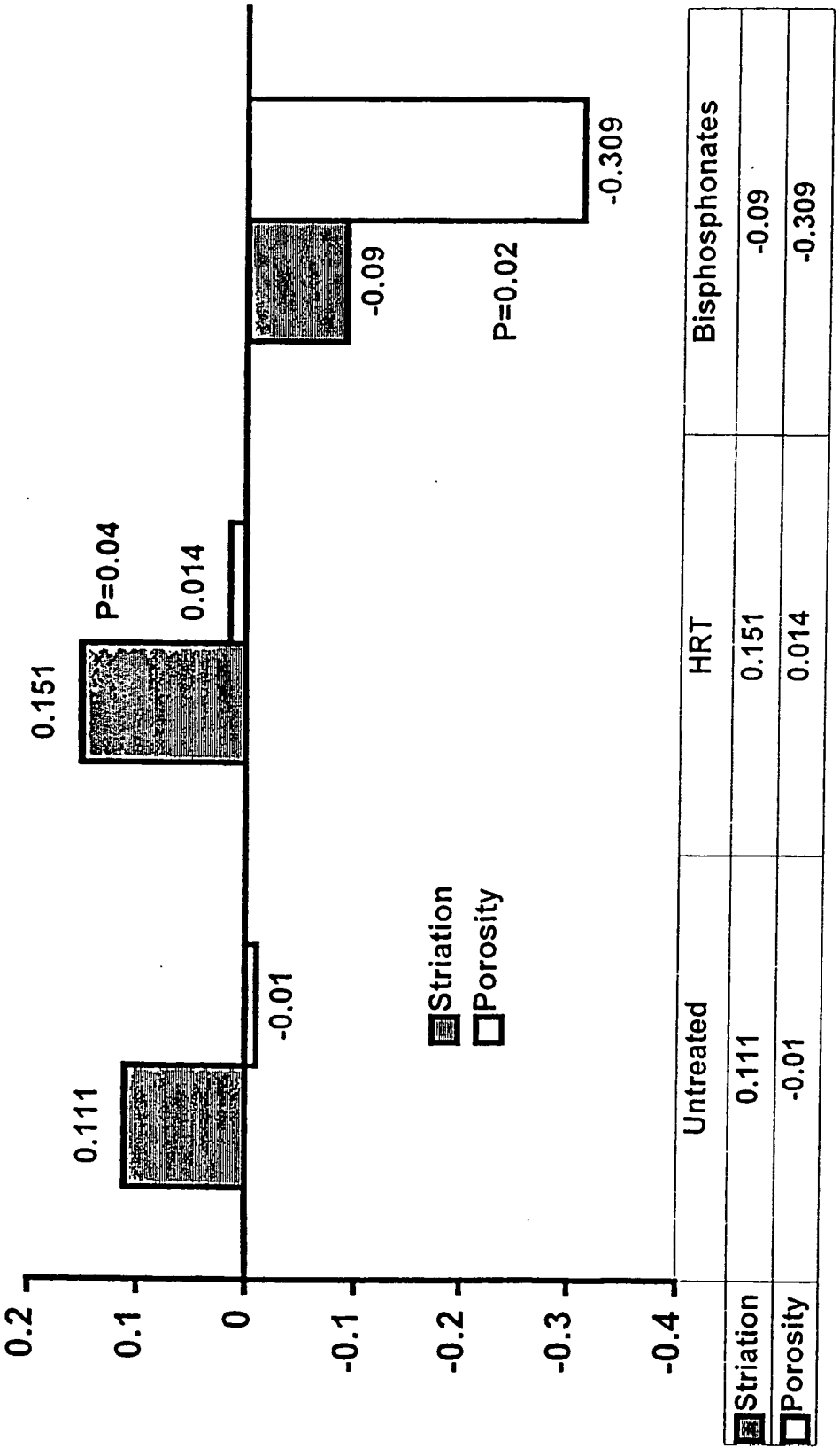
Annual changes expressed in percentages for the bisphosphonate group

Fig. 5



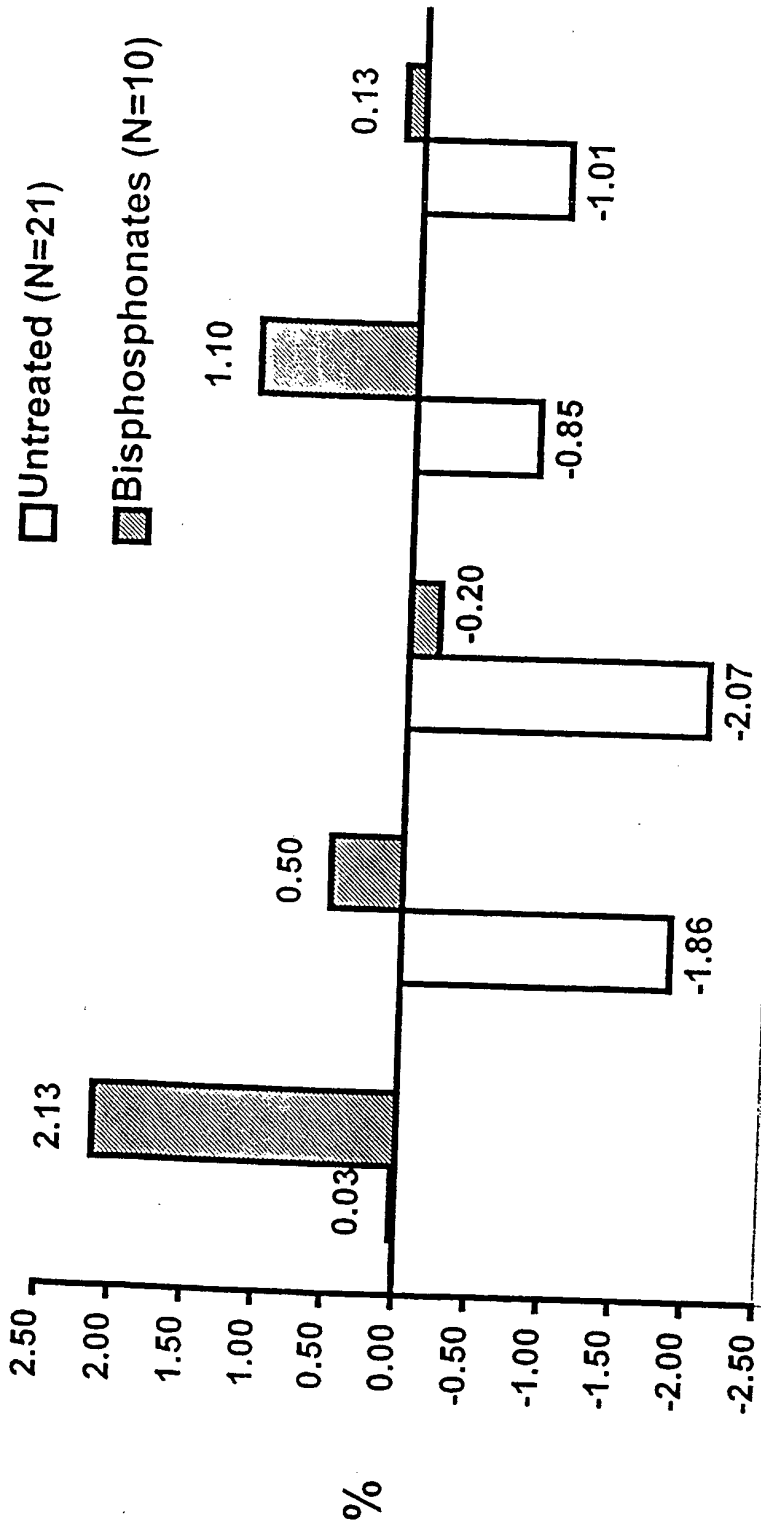
SD normalized annual changes for the bisphosphonate group (n=11)

Fig. 6



The actual annual changes for porosity and striation

Fig. 7



	DXA Spine	DXA Hip	DXA Forearm	DXR	Metac. T. (2)
Untreated (N=21)	0.03	-1.86	-2.07	-0.85	-1.01
Bisphosphonates (N=10)	2.13	0.50	-0.20	1.10	0.13

Annual changes expressed in percentages for the subsample of untreated and bisphosphonate treated women

Fig. 8

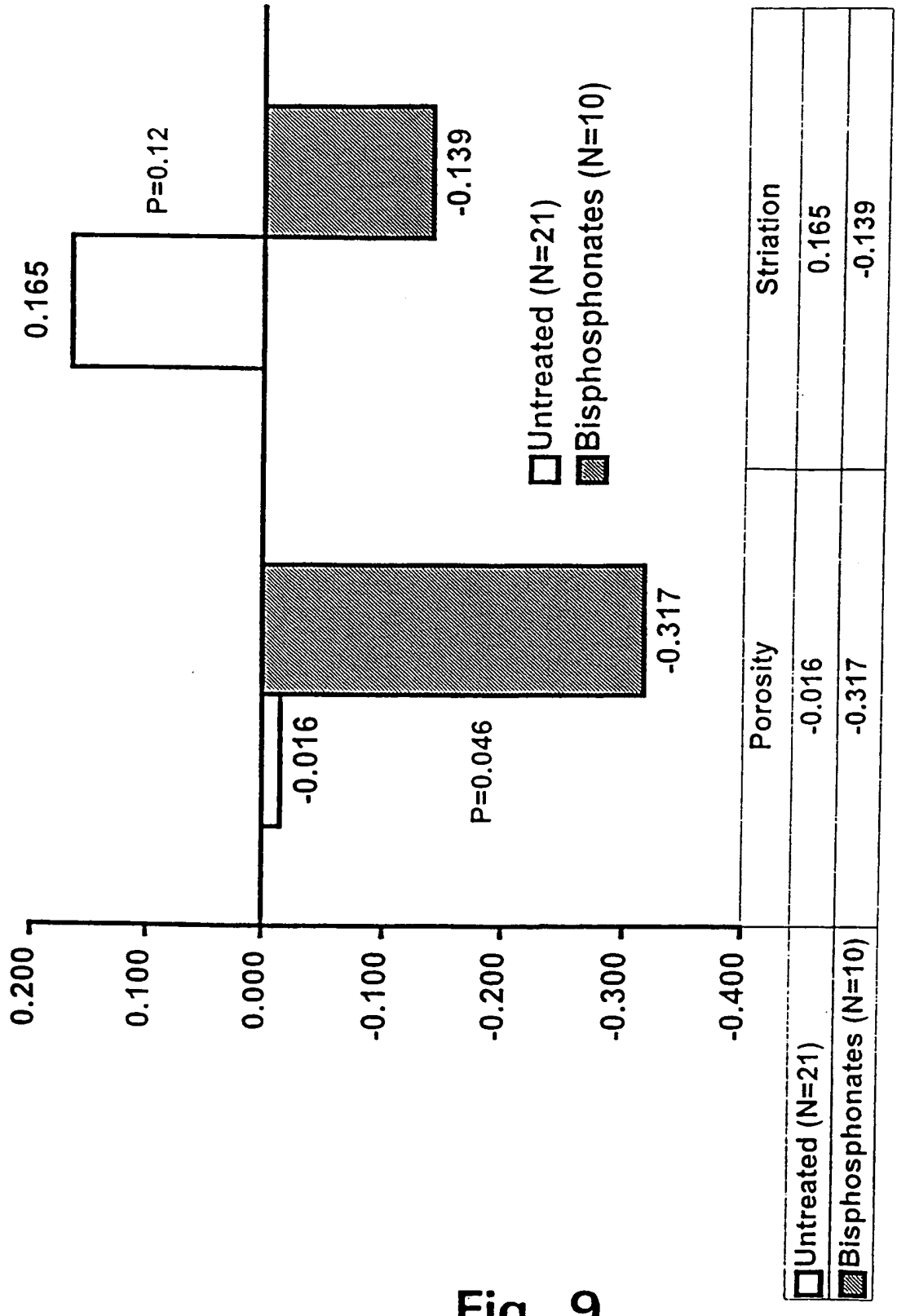


Fig. 9

Figure 9. The actual annual changes for porosity and striation for the subsample of untreated and bisphosphonate treated women

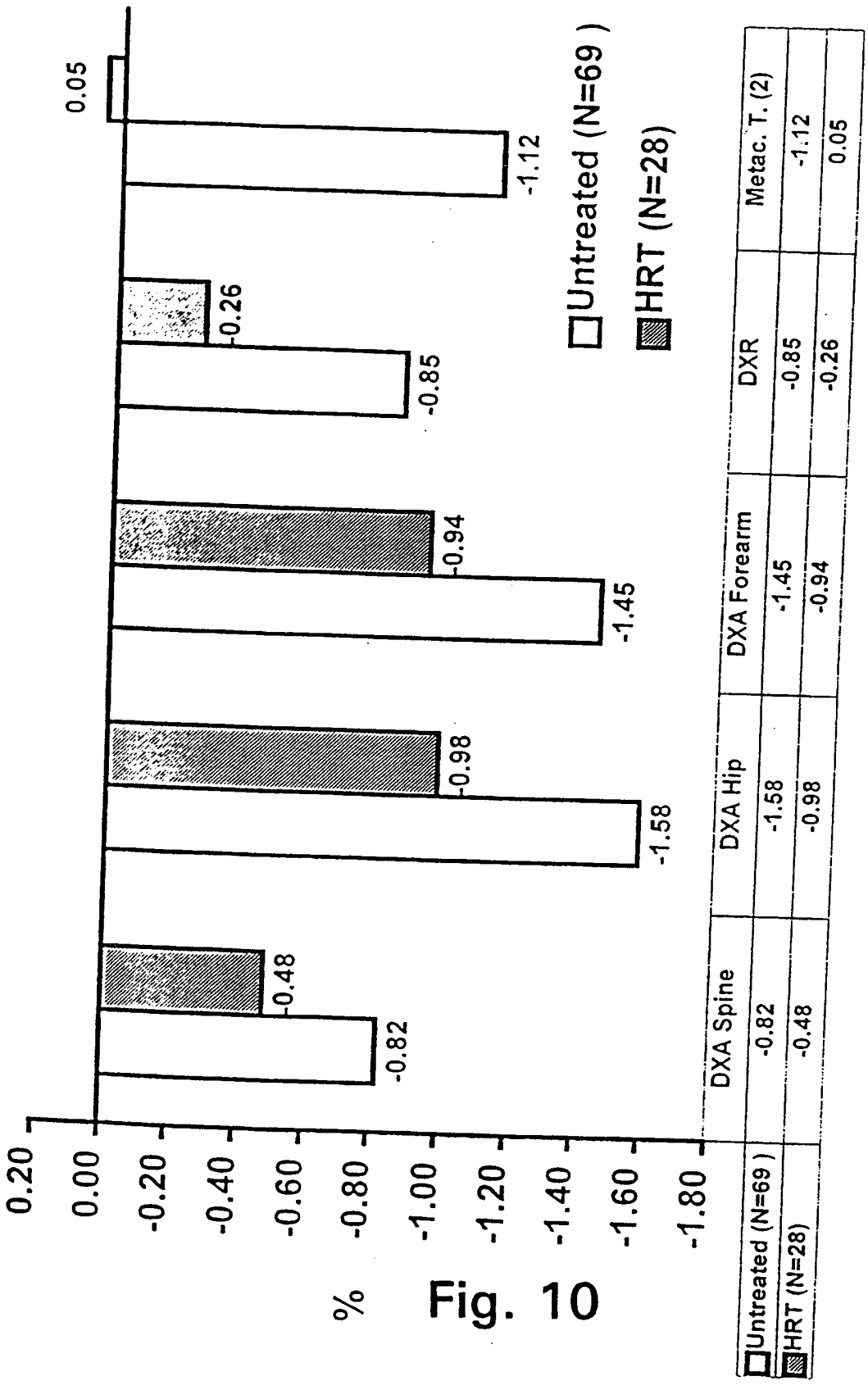
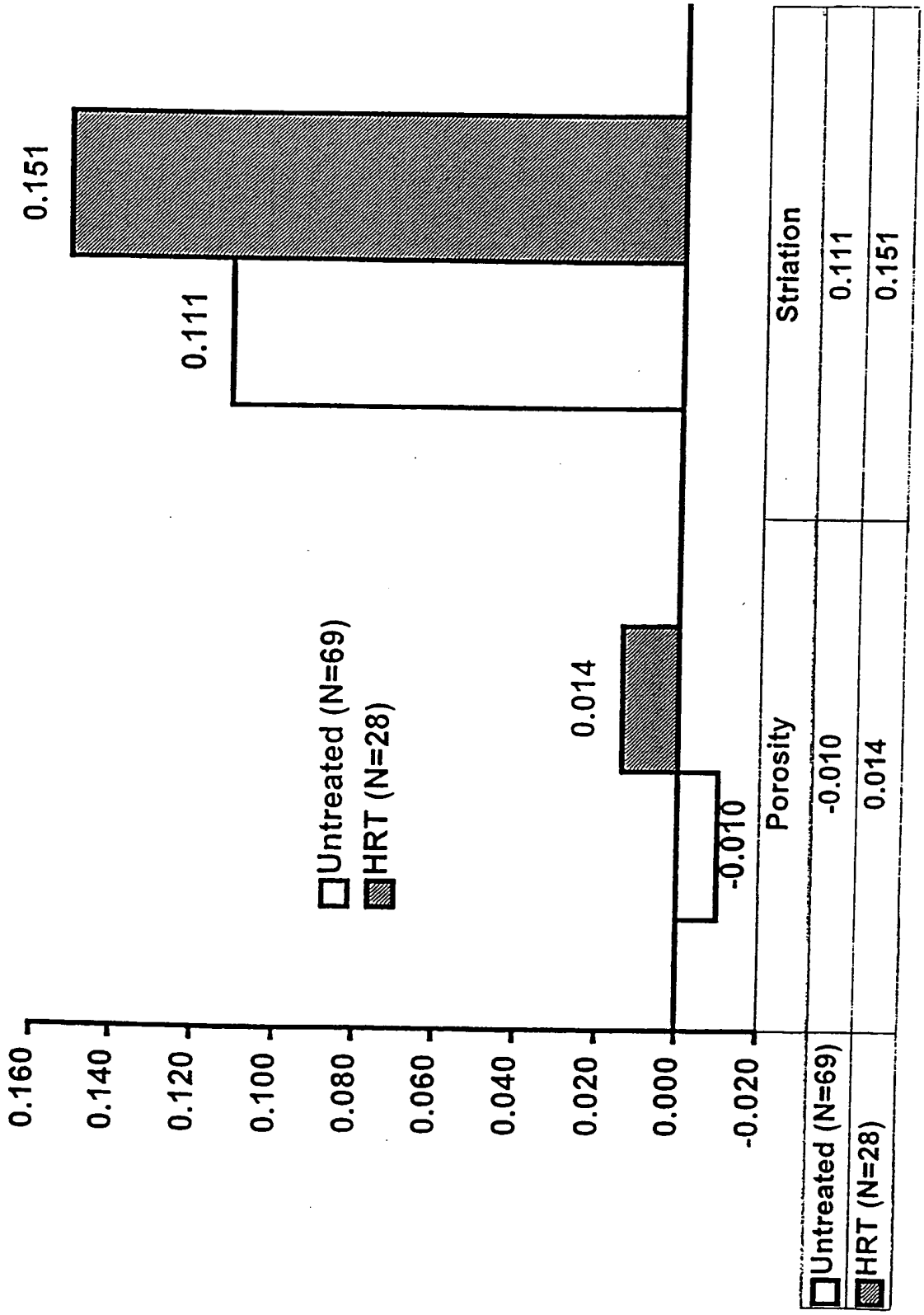


Fig. 10

Annual changes expressed in percentages for the untreated and the "HRT>90%" treated group.



The actual annual changes for porosity and striation

Fig. 11

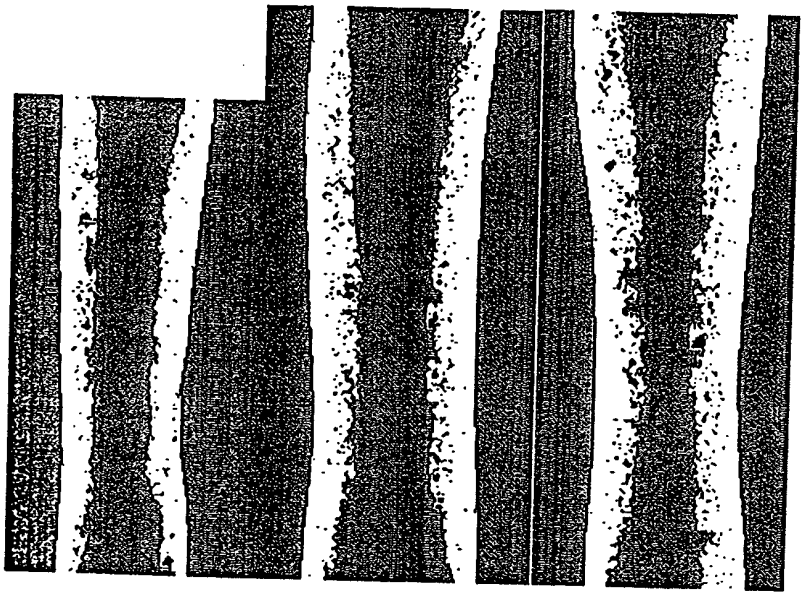
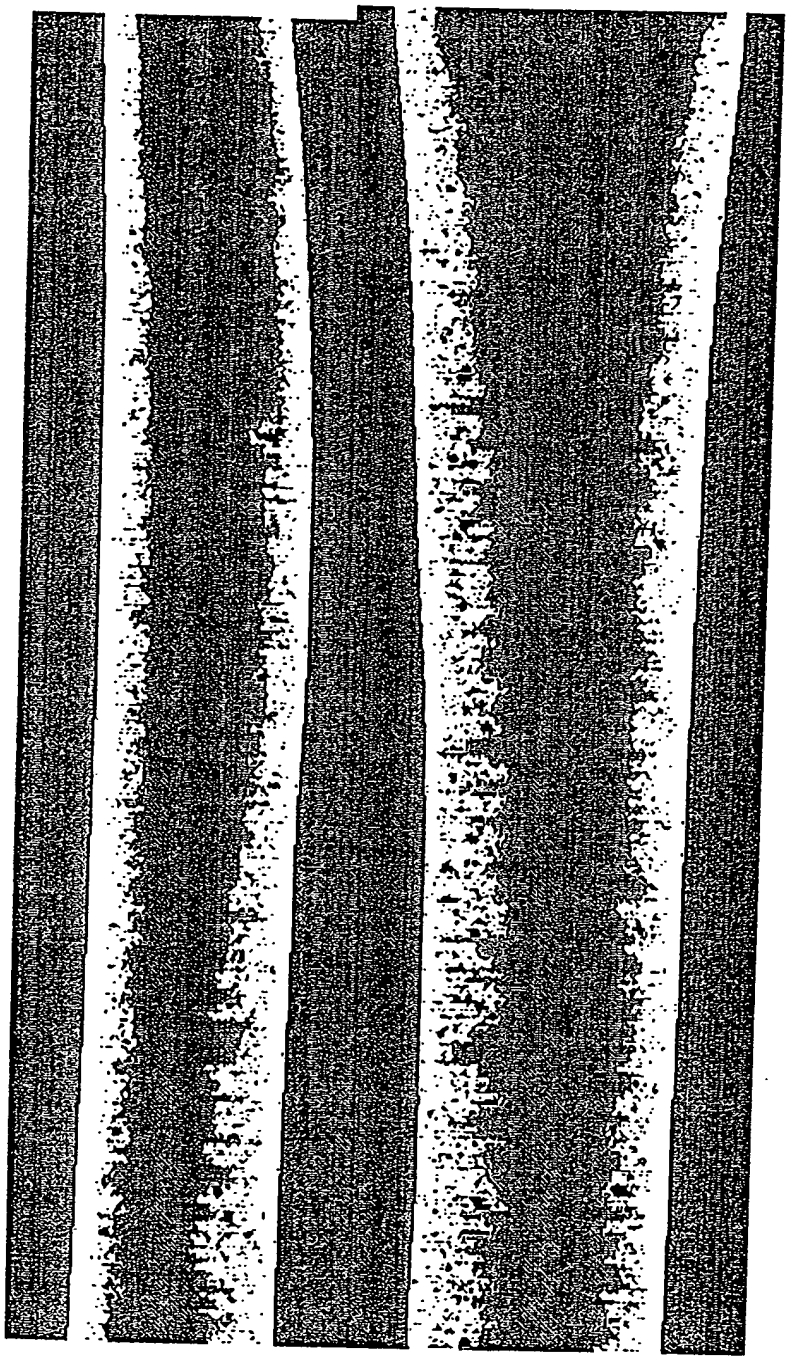


Fig. 12



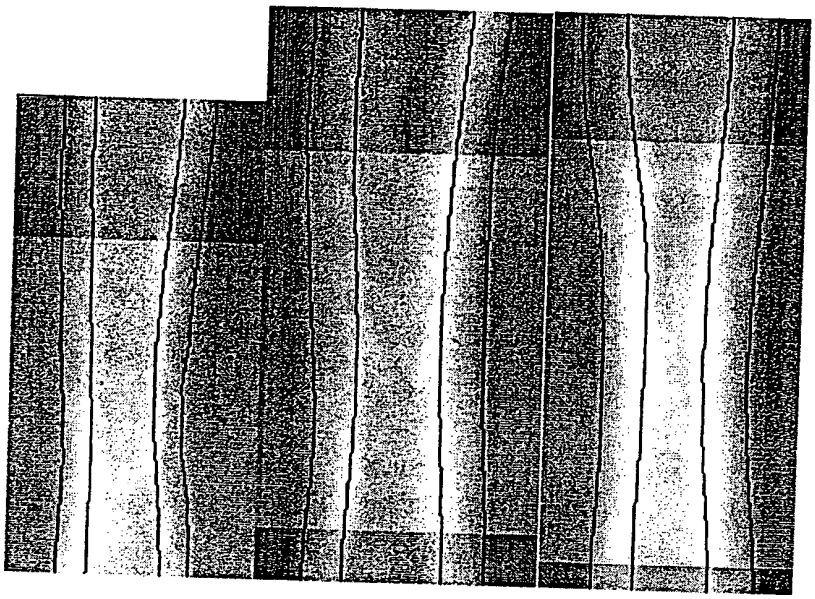
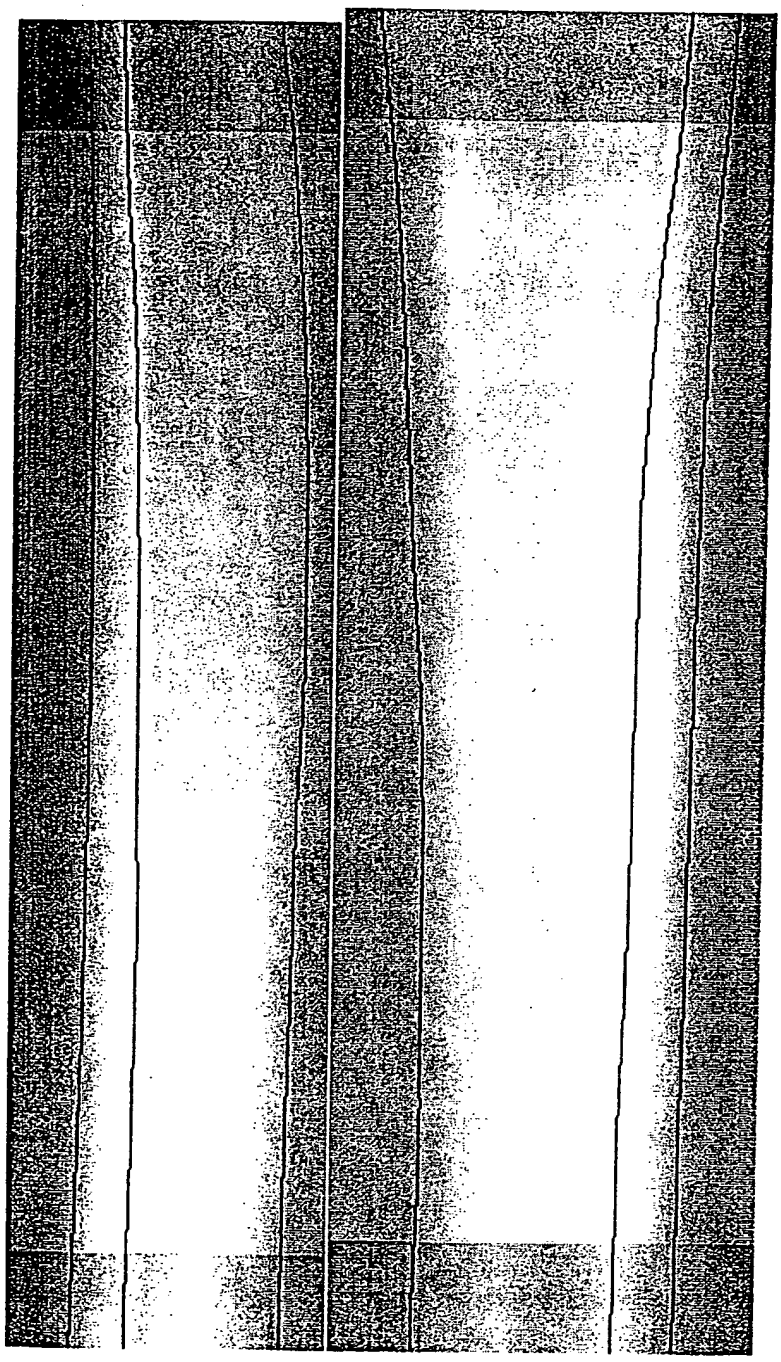


Fig. 13



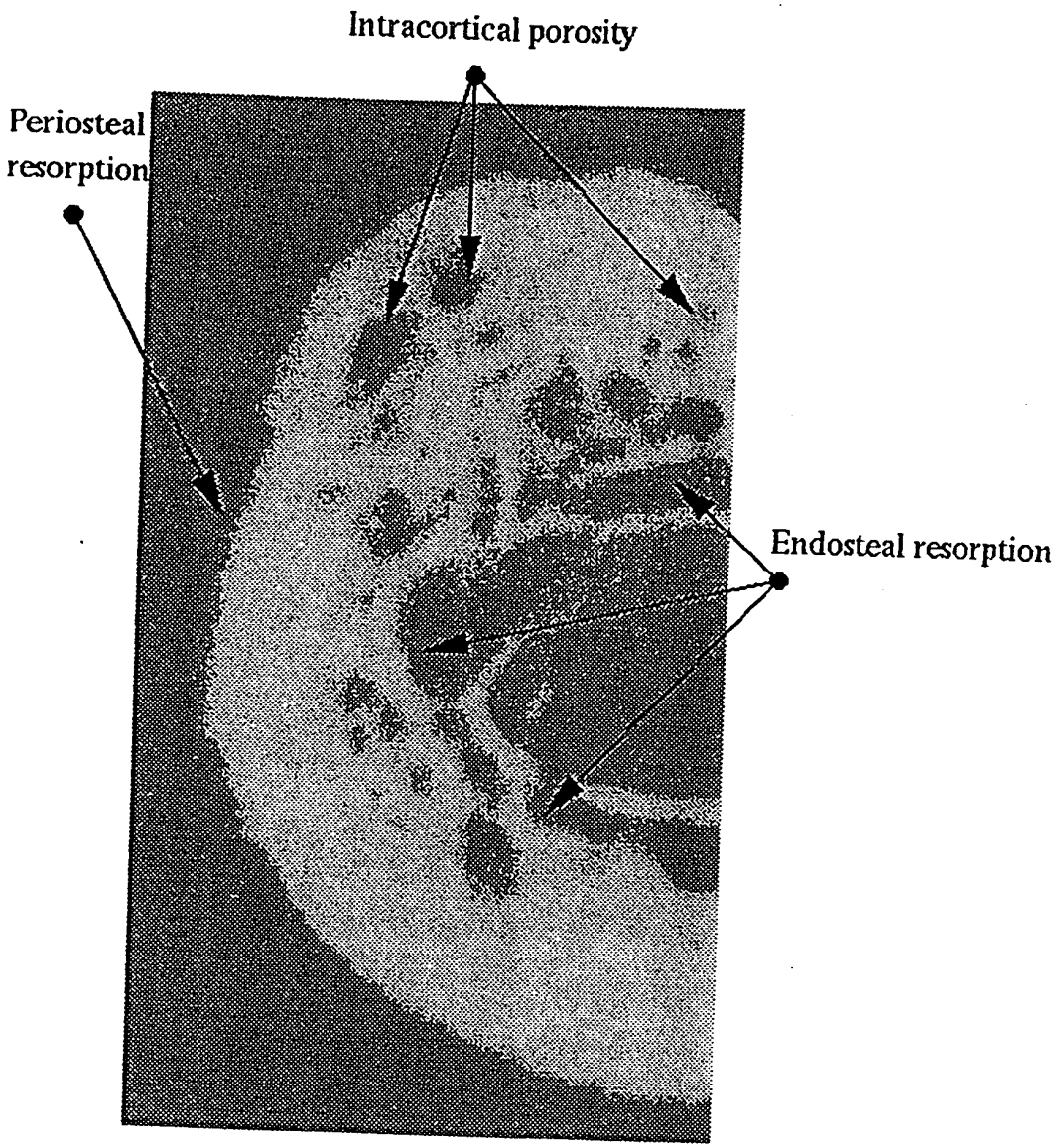

Intracortical porosity
Periosteal resorption
Endosteal resorption

Fig. 14

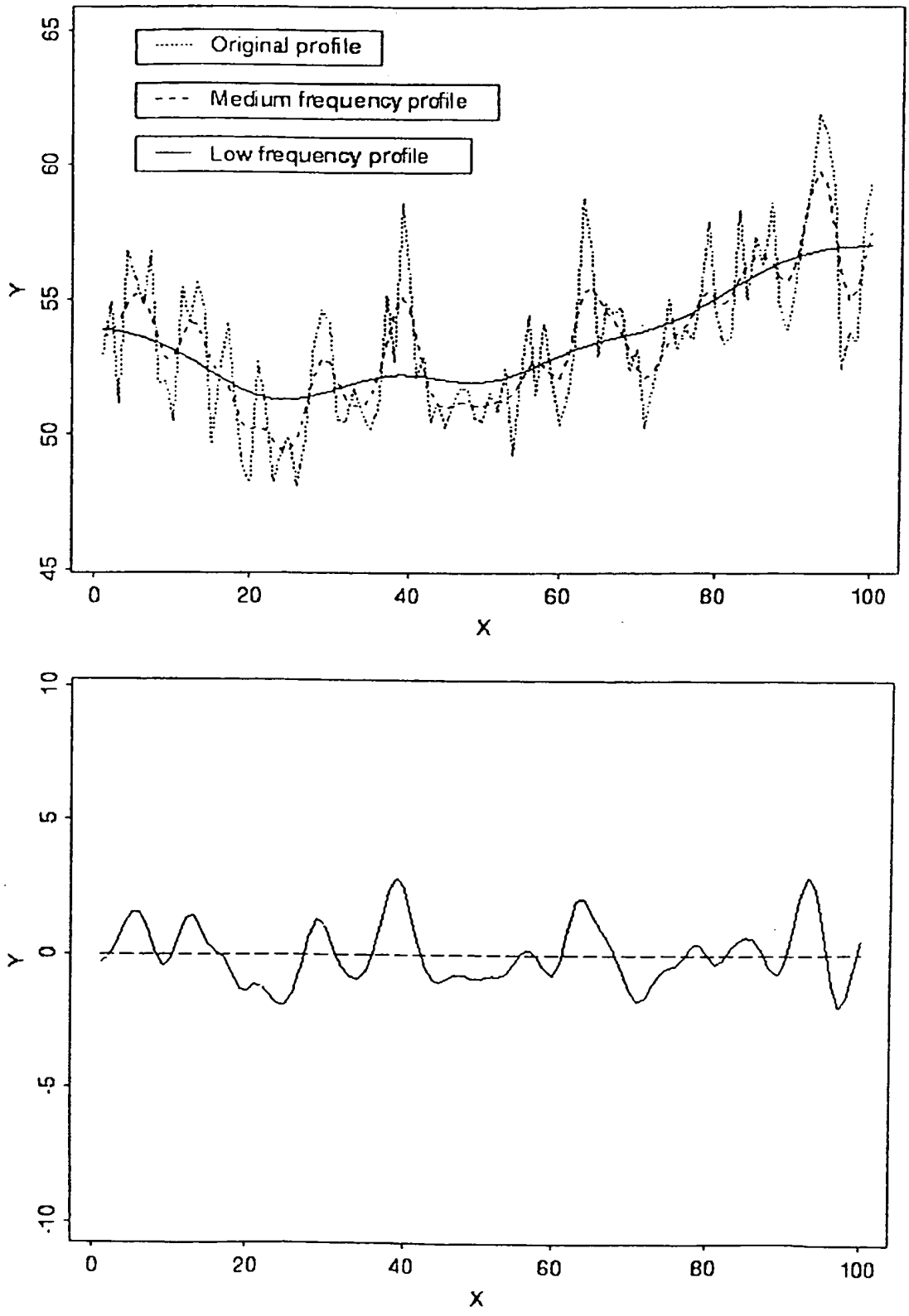

Original profile
Medium frequency profile
Low frequency profile
Y
X
65
60
55
50
45
0
20
40
60
80
100
10
5
0
-5
-10

Fig. 15

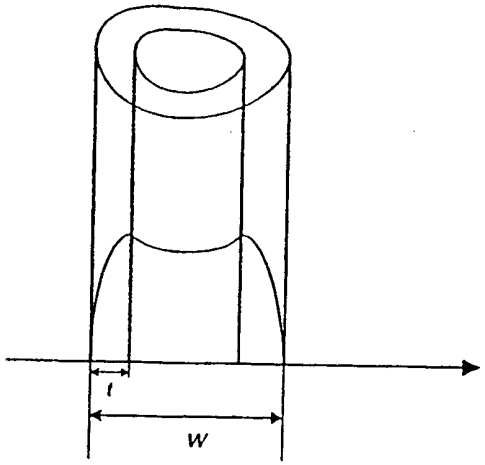


Fig. 16

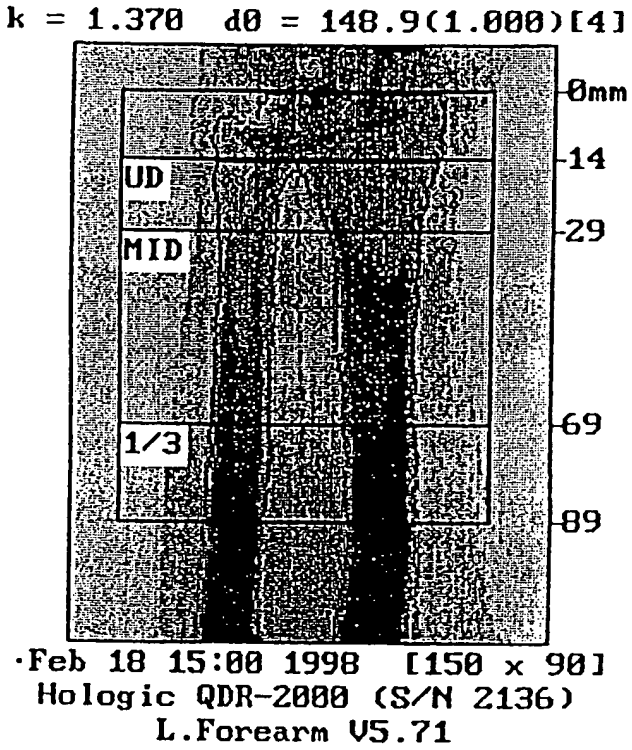

k = 1.370 d0 = 148.9(1.000)[4]
0mm
14
29
69
89
UD
MID
1/3
·Feb 18 15:00 1998 [150 x 90]
Hologic QDR-2000 (S/N 2136)
L.Forearm V5.71

Fig. 17

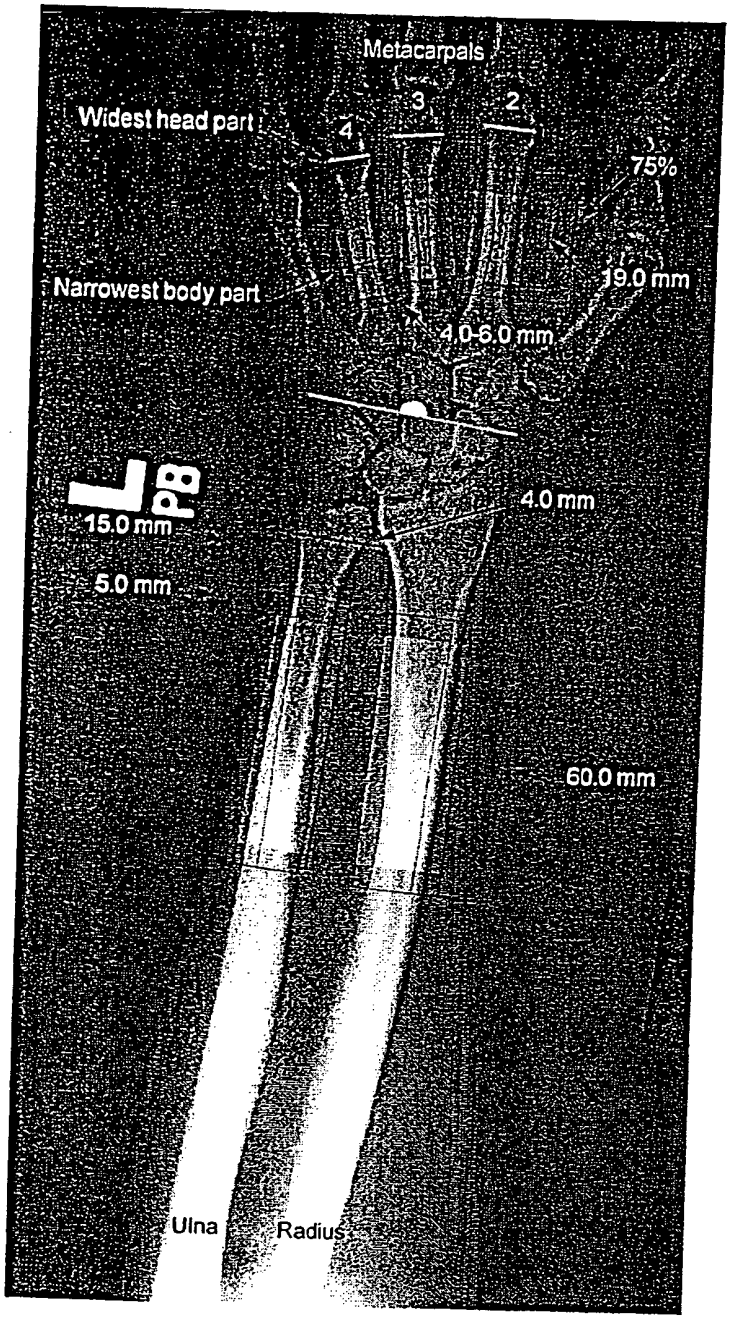

Metacarpals
3
2
Widest head part
4
75%
19.0 mm
Narrowest body part
4.0-6.0 mm
PB
15.0 mm
4.0 mm
5.0 mm
60.0 mm
Ulna
Radius

Fig. 18

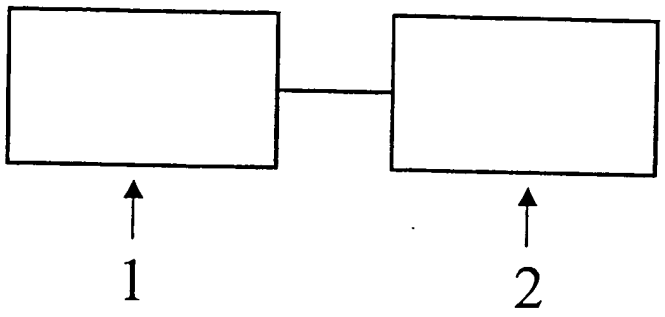


Fig. 19

INTERNATIONAL SEARCH REPORT

Internat'l Application No
PCT/uK 00/00273

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G06F19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G06F A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 247 934 A (WEHRLI FELIX W ET AL) 28 September 1993 (1993-09-28) column 3, line 33 -column 5, line 9 column 7, line 9 - line 42 column 10, line 25 - line 61; figures 3,4 ---	1-115
X	US 5 228 068 A (MAZESS RICHARD B) 13 July 1993 (1993-07-13) column 2, line 62 -column 3, line 37 column 12, line 19 - line 58; figures 1,5 ---	1-115
A	EP 0 905 638 A (TORSANA A S) 31 March 1999 (1999-03-31) abstract ---	1-115
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 August 2000	15 09 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	C. Lyckman

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/UK 00/00273

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 721 112 A (HIRANO YOSHIO ET AL) 26 January 1988 (1988-01-26) column 1, line 56 -column 2, line 53 abstract -----	1-115

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK00/00273

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-104
because they relate to subject matter not required to be searched by this Authority, namely:
See next sheet.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK00/00273

See PCT Rule 39.1(iv)
Method for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
Nevertheless, a search has been executed for these claims. The search has been based on the method implemented in an apparatus.

INTERNATIONAL SEARCH REPORT

In ...nation on patent family members

Internat ...al Application No
PCT/UK 00/00273

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